FORM PTO 1390 (REV 9-2001) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		HO-P02428US0
		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/089928 Unknown
INTERNATIONAL APPLICATION NO. PCT/GB00/03800	INTERNATIONAL FILING DATES October 4, 2000	PRIORITY DATE CLAIMED October 4, 1999
TITLE OF INVENTION UTROPHIN GENE PROMOTER		
APPLICANT(S) FOR DO/EO/US Edward Burton, et al.		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing 35 U.S.C. 371
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371 (f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. [x] A copy of the International Application as filed (35 U.S.C. 371 (c)(2))
   a. [x] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [x] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [x] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [x] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [ ] Other items or information: **SEE ATTACHED RETURN POSTCARD**

10089928 .092702

JC13 Rec'd PCT/PTO 04 APR 2002

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/089928 Unknown	PCT/GB00/03800	HO-P02428US0

21. [x] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) – (5)):

		CALCULATIONS PTO USE ONLY
[ ]	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040.00	
[x]	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00	
[ ]	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $740.00	
[ ]	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00	
[ ]	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00
	Surcharge of $ 130.00 for furnishing the oath or declaration later than [ ] 20 [x] 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$ 130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	113-20	93	x 18.00	$	1,674.00
Independent claims	15-3	12	x 84.00	$	1,008.00
MULTIPLE DEPENDENT CLAIM(s) (if applicable)			+ 280.00	$	280.00
			TOTAL OF ABOVE CALCULATIONS =	$	3,982.00
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				$	
			SUBTOTAL =	$	3,982.00
Processing fee of $ _____ for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				$	
			TOTAL NATIONAL FEE =	$	3,982.00
Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) + (per property).				$	
			TOTAL FEES ENCLOSED =	$	3,982.00
			Amount to be Refunded:	$	
			Charged:	$	

a. [x] A check in the amount of $ 3,982.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [x] The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to my Deposit Account No. 06-2375. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Melissa W. Acosta
FULBRIGHT & JAWORSKI L.L.P.
1301 McKinney, Suite 5100
Houston, Texas 77010-3095
(713) 651-5407

SIGNATURE

Melissa W. Acosta
NAME

45,872
REGISTRATION NUMBER

10/089928

JC13 Rec'd PCT/PTC 0 4 APR 2002

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EU098493497US, in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on the date shown below.

Dated: April 4, 2002 Signature: Staci Harris (Staci Harris)

Docket No.: HO-P02428US0 (PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Edward Burton, et al.

Application No.: Unknown Group Art Unit:

Filed: April 4, 2002 Examiner:

For: UTROPHIN GENE PROMOTER

STATEMENT PURSUANT TO 37 CFR 1.823(b))

U.S. Patent and Trademark Office
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Submitted herewith for filing in connection with the above-referenced patent application is a labeled, computer readable copy of the Sequence Listing included in the application.

I hereby state that I have reviewed the paper copy of the Sequence Listing contained, as required by 37 CFR 1.821(e), and the computer readable form of the Sequence Listing, as required by 37 CFR 1.821(c), and that the content of the paper and computer readable copies are the same.

Early favorable consideration of the patent application is respectfully solicited.

Dated: April 4, 2002

Respectfully submitted,

By _____
Melissa W. Acosta
Registration No.: 45,872
FULBRIGHT & JAWORSKI L.L.P.
1301 McKinney, Suite 5100
Houston, Texas 77010-3095
(713) 651-5407
(713) 651-5246 (Fax)

25152728.1

Docket No.: HO-P02428US0

(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Edward Burton, et al.

Application No.: UNKNOWN

Group Art Unit: N/A

Filed: April 4, 2002

Examiner: Not Yet Assigned

For: UTROPHIN GENE PROMOTER

FIRST PRELIMINARY AMENDMENT

Box Non-Fee Amendment
Commissioner for Patents
Washington, DC 20231
Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

In the Claims

Please add the following new claims 53-111.

53. An isolated nucleic acid comprising a promoter which comprises a sequence of nucleotides selected from (i) the human promoter sequence shown in Figure 1 and (ii) the mouse promoter sequence shown in Figure 2, free or substantially free of utrophin coding sequence.

54. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1440 in Figure 1.

55. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1183 of the mouse sequence shown in Figure 2.

56. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 1199 -1440 in the sequence shown in Figure 1.

57. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 959-1183 in the sequence shown in Figure 2.

58. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotide sequence ACAGGACATCCCAGTGTGCAGTTCG.

59. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 1, which sequence has at least 60% homology with the promoter sequence shown in figure 1 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

60. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which sequence has at least 60% homology with the promoter sequence shown in figure 2 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

61. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which hybridises to the promoter sequence shown in figure 2 under stringent hybridisation conditions and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

62. A nucleic acid construct comprising an isolated nucleic acid according to any of the preceding claims operably linked to a heterologous sequence.

63. A nucleic acid construct comprising an isolated nucleic acid according to any one of claims 53 to 61 operably-linked to a coding sequence.

64. A nucleic acid construct according to claim 63 wherein said coding sequence encodes a reporter molecule.

65. An in vitro host cell comprising a nucleic acid construct according to claim 63.

66. An in vitro host cell comprising a nucleic acid construct according to claim 64.

67. A method comprising culturing a host cell according to claim 65 under conditions for expression of the peptide or polypeptide encoded by said coding sequence.

68. A method as claimed in claim 67 wherein said coding sequence encodes a reporter molecule.

69. A method according to claim 67 comprising detection of transcription of said coding sequence.

70. A method according to claim 67 comprising detection of expression of the peptide or polypeptide encoded by said coding sequence.

71. A method of screening for a substance able to modulate utrophin promoter activity, the method comprising contacting an expression system containing a nucleic acid construct according to claim 63 with a test or candidate substance and determining transcription of said coding sequence or expression of the peptide or polypeptide encoded by said coding sequence.

72. A method as claimed in claim 63 wherein said coding sequence encodes a reporter molecule and said reporter molecule is detected.

73. A method according to claim 71 wherein the expression system comprises a host cell containing said nucleic acid construct.

74. A method which comprises, following identification of a substance able to modulate utrophin promoter activity in accordance with a method according to claim 71, manufacture of the substance and/or use of the substance in manufacture or formulation of a composition.

75. The use of an isolated nucleic acid according to any of claims 53 to 58 for promoting transcription of an operably linked sequence of nucleotides.

76. The use of claim 75 wherein the transcription is tissue-specific, with the tissue-specificity being muscle-specific.

77. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide including the amino acid sequence shown in Figure 1 or Figure 2.

78. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide including the amino acid sequence shown in Figure 1, which amino acid sequence has at least 60% homology with the polypeptide sequence in Figure 1 or Figure 2.

79. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide shown in Figure 1 or Figure 2, which nucleotide sequence hybridises with the nucleotide sequence encoding the polypeptide in Figure 1 or Figure 2 under stringent hybridisation conditions.

80. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence shown in Figure 9.

81. An isolated nucleic acid molecule comprising the nucleotide sequence shown in Figure 9.

82. A nucleic acid of any one of claims 77 to 81 comprised in a vector.

83. A nucleic acid according to any one of claims 77 to 81 comprised in an expression vector.

84. An in vitro host cell containing an expression vector according to claim 83.

85. A method including introduction of nucleic acid according to any of claims 77 to 81 into a cell.

86. A method as claimed in claim 85 wherein said nucleic acid is an expression vector.

87. A method according to claim 85 wherein said introduction takes place in vitro.

88. A method as claimed in claim 85 which includes causing or allowing expression of said polypeptide encoding nucleotide sequence in a cell.

89. A method according to claim 88 wherein the cell is part of a mammal.

90. A method according to claim 88 wherein the expression product is purified and/or isolated following expression.

91. A method according to claim 90 wherein the expression product is formulated into a composition which includes at least one additional component, following purification and/or isolation of the expression product.

92. An isolated polypeptide as encoded by nucleic acid according to any of claims 77 to 81.

93. An isolated utrophin exon IB polypeptide selected from:

(i) human utrophin exon IB polypeptide of which the amino acid sequence is shown in Figure 1; and

(ii) mouse utrophin exon IB of which the amino acid sequence is shown in Figure 1.

94. An isolated polypeptide including the human polypeptide according to claim 93.

95. An isolated polypeptide including the mouse polypeptide according to claim 93.

96. An isolated polypeptide which has 60 % homology with the polypeptide according to claim 94 or 95.

97. An isolated fragment of a polypeptide according to claim 93, which fragment is 5 to 25 amino acids in length.

98. An isolated fragment of a polypeptide according to claim 93, which fragment is 10 to 20 amino acids in length.

99. An antibody specific for a polypeptide according to any one of claims 92 to 96.

100. A composition including a polypeptide according to claim 92 and a pharmaceutically acceptable excipient.

101. A composition including a polypeptide according to any one of claims 92 to 98 and a pharmaceutically acceptable excipient.

102. A composition including a polypeptide according to claim 94 and a pharmaceutically acceptable excipient.

103. A composition including a fragment according to claim 97 or claim 98 and a pharmaceutically acceptable excipients.

104. A composition including an antibody according to claim 99 and a pharmaceutically acceptable excipients.

105. A method for treating a dystrophin phenotype in a mammal, which comprises administering a nucleic acid according to any one of claims 77 to 81 in a therapeutically effective amount.

106. A method as claimed in claim 105 wherein said nucleic acid is an expression vector.

107. A method for treating a dystrophin phenotype in a mammal, which comprises administering a polypeptide according to claim 92 in a therapeutically effective amount.

108. A method for treating a dystrophin phenotype in a mammal, which comprises administering a polypeptide according to any one of claims 93 to 95 in a therapeutically effective amount.

109. A method for treating a dystrophin phenotype in a mammal, which comprises administering a polypeptide according to claim 96 in a therapeutically effective amount.

110. A method for treating a dystrophin phenotype in a mammal, which comprises administering a fragment according to claim 97 or claim 98 in a therapeutically effective amount.

111. A method for treating a dystrophin phenotype in a mammal, which comprises administering an antibody according to claim 99 in a therapeutically effective amount.

Application No.: UNKNOWN Docket No.: HO-P02428US0

REMARKS/ARGUMENTS

Claims 1-52 were in the original PCT application as filed. Applicants have canceled claims 53-111, without prejudice or acquiescence and have added claims 53-111. Claims 53-111 delete the multiple dependency and clarify the claims without prejudice or acquiescence. Applicants assert that no new matter has been added.

CONCLUSION

Applicants have added claims 53-111 to delete the multiple dependency and to clarify the claims without prejudice or acquiescence. Claims 53-111 have been canceled without prejudice or acquiescence. Therefore, these amendments do not narrow the scope of the claims within the meaning of *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.*, 234 F.3d 558, 586, 56 USPQ2d 1865, 1886 (Fed. Cir. 2000).

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Dated: April 4, 2002

Respectfully submitted,

By _____

Melissa W. Acosta
Registration No.: 45,872
FULBRIGHT & JAWORSKI L.L.P.
1301 McKinney, Suite 5100
Houston, Texas 77010-3095
(713) 651-5151
(713) 651-5246 (Fax)

UTROPHIN GENE PROMOTER

The present invention is based on cloning of a genomic promoter region of the human utrophin gene and of the mouse utrophin gene.

The severe muscle wasting disorders Duchenne muscular dystrophy (DMD) and the less debilitating Becker muscular dystrophy (BMD) are due to mutations in the dystrophin gene resulting in a lack of dystrophin or abnormal expression of truncated forms of dystrophin, respectively. Dystrophin is a large cytoskeletal protein (427kDa with a length of 125nm) which in muscle is located at the cytoplasmic surface of the sarcolemma, the neuromuscular junction (NMJ) and myotendinous junction (MTJ). It binds to a complex of proteins and glycoproteins spanning the sarcolemma called the dystrophin associated glycoprotein complex (DGC). The breakdown of the integrity of this complex due to loss of, or impairment of dystrophin function, leads to muscle degeneration and the DMD phenotype.

The dystrophin gene is the largest gene so far identified in man, covering over 2.7 megabases and containing 79 exons. The corresponding 14kb dystrophin mRNA is expressed predominantly in skeletal, cardiac and smooth muscle with lower levels in brain. Transcription of dystrophin in different tissues is regulated from either the brain promoter (predominantly active in neuronal cells) or muscle promoter (differentiated myogenic cells, and primary glial cells) giving rise to differing first exons. A third promoter between the muscle promoter and the second exon of dystrophin regulates expression in cerebellar Purkinje neurons. Recently reviewed in (Tinsley, *et al* (1994) *Proc Natl Acad Sci U S A* **91**, 8307-13, Blake, *et al* (1994) *Trends in Cell Biol.* **4**: 19-23,Tinsley, *et al* (1993) *Curr Opin Genet Dev.* **3**: 484-90).

There are various approaches which have been adopted for the gene therapy of DMD, using the *mdx* mouse as a model system. However, there are considerable problems related to the number of muscle cells that can be made dystrophin positive, the levels of expression of the gene and the duration of expression (Partridge, *et al.* (1995) *British Medical Bulletin* **51**: 123-137). It has also become apparent that simply re-introducing genes expressing the dystrophin carboxy-terminus has no effect on the dystrophic phenotype although the DGC appears to be re-established at the sarcolemma (Cox, *et al.* (1994) *Nature Genet* **8**: 333-339,Greenberg, *et al.* (1994) *Nature Genet* **8**: 340-344).

In order to circumvent some of these problems, possibilities of compensating for dystrophin loss using a related protein, utrophin, are being explored as an alternative route to dystrophin gene therapy. A similar strategy is currently being evaluated in clinical trials to up-regulate foetal haemoglobin to compensate for the affected adult-globin chains in patients with sickle cell anaemia (Rodgers, *et al.* (1993) *N Engl J Med.* **328**: 73-80,Perrine, *et al.* (1993) *N Engl J Med.* **328**: 81-86).

Utrophin is a 395kDa protein encoded by multiexonic 1Mb *UTRN* gene located on chromosome 6q24 (Pearce, *et al.* (1993) *Hum Mol Gene.* **2**: 1765-1772). At present the tissue regulation of utrophin is not fully understood. In the dystrophin deficient *mdx* mouse, utrophin levels in muscle remain elevated soon after birth compared with normal mice; once the utrophin levels have decreased to the adult levels (about 1 week after birth), the first signs of muscle fibre necrosis are detected. However there is evidence to suggest that in the small calibre muscles, continual increased levels of utrophin can interact with the DGC complex (or an antigenically related complex) at the sarcolemma thus preventing loss of the complex with the result that these muscles appear normal. There is also a

substantial body of evidence demonstrating that utrophin is capable of localising to the sarcolemma in normal muscle. During fetal muscle development there is increased utrophin expression, localised to the sarcolemma, up until 18 weeks in the human and 20 days gestation in the mouse. After this time the utrophin sarcolemmal staining steadily decreases to the significantly lower adult levels shortly before birth where utrophin is localised almost exclusively to the NMJ. The decrease in utrophin expression coincides with increased expression of dystrophin. See reviews (Ibraghimov Beskrovnaya, *et al.* (1992) *Nature* **355**, 696-702.,Blake, *et al.* (1994) *Trends in Cell Biol*,.**4**: 19-23,Tinsley, *et al.* (1993) *Curr Opin Genet Dev.* **3**: 484-90).

Thus, in certain circumstances utrophin can localise to the sarcolemma probably at the same binding sites as dystrophin, through interactions with actin and the DGC. Accordingly, if expression of utrophin is sufficiently elevated, it may maintain the DGC and thus alleviate muscle degeneration in DMD/BMD patients (Tinsley, *et al.* (1993) *Neuromuscul Disord* **3**, 537-9.).

However, manipulation of utrophin expression and screening for molecules able to upregulate expression is hampered by the limited understanding of utrophin expression regulation and its promoters. We have previously isolated a promoter element lying within the CpG island at the 5' end of the utrophin locus that is active in a broad range of cell types and tissues, and shown it to be synaptically regulated *in vivo* (Dennis, et al. (1996) *Nucleic Acids Res* **24**, 1646-52 and WO 96/34101). The sequence contains a consensus N-box, a 6bp motif important in the regulation of other genes expressed at the NMJ (Koike, *et al.* (1995) *Proc Natl Acad Sci USA* **92**, 10624-10628). Localisation of utrophin at the NMJ in mature muscle is partially attributable to enhanced transcription of utrophin at sub-junctional myonuclei, with consequent synaptic

accumulation of mRNA (Gramolini, *et al.* (1997) *J Biol Chem* **272**, 8117-20, Vater, *et al.* (1998) *Molecular and Cellular Neuroscience* **10**, 229-242). The utrophin promoter drives synaptic transcription of a reporter gene in *vivo*; this expression pattern is abolished by point mutations within the N-box (Gramolin, et al. (1998) J Biol Chem **273**, 736-43).

The present inventors hypothesised that utrophin might be transcribed from more than one promoter, an important consideration for the following reasons: First, it may be undesirable to interfere with the mechanisms underlying synaptic regulation of genes, as this might affect expression of other post-synaptic components and impair the structure and function of the NMJ; a promoter without synaptic regulatory elements might be a more suitable target for pharmacological manipulation. Second, cardiac dysfunction is a common feature of the dystrophinopathies (Hoogerwaard, *et al.* (1997) *J Neurol* **244**, 657-63, Sasaki, *et al.* (1998) *Am Heart J* **135**, 937-44); if the cardiac utrophin message was transcribed from a different promoter, then it might prove necessary to up-regulate this. Finally, inclusion of additional regulatory sequences might increase the yield of a screening program to identify small molecules capable of transcriptional activation of utrophin.

We have now identified an alternative promoter lying within the large second intron of the utrophin gene, 50kb 3' to exon 2. The promoter is highly regulated, expressed in a wide range of tissues and has little similarity to the synaptically expressed promoter. This promoter drives transcription of a widely expressed unique first exon that splices into a common full-length mRNA at exon 3. This unique exon (called exon IB) encodes a novel 31 amino acid N-terminus for the utrophin protein which may be involved in binding to the muscle membrane. The sequences of the two utrophin promoters are dissimilar, and we predict that they respond to discrete sets of cellular signals.

Exon IB is primarily considered herein to encode the indicated 31 amino acids. However, the splice occurs within a codon for aspartate. This aspartate residue is common to both isoforms of utrophin. In embodiments of the invention an aspartate residue may be included C-terminal to the 31 amino acids to provide a 32 amino acid peptide, which may be joined to additional amino acids, for instance additional utrophin sequence as discussed. See, for instance, Figure 8 for one embodiment.

These findings significantly contribute to the understanding of the molecular physiology of utrophin expression and are important because the promoter reported here provides an alternative target for transcriptional activation of utrophin in DMD muscle. This promoter does not contain synaptic regulatory elements and might, therefore, be a more suitable target for pharmacological manipulation than the previously described promoter.

We have now cloned this alternative utrophin promoter and exon, and the present invention in various aspects and embodiments is based on the sequence information obtained and provided herein.

One major use of the promoter is in screening for substances able to modulate its activity. It is well known that pharmaceutical research leading to the identification of a new drug generally involves the screening of very large numbers of candidate substances, both before and even after a lead compound has been found. This is one factor which makes pharmaceutical research very expensive and time-consuming. A method or means assisting in the screening process will have considerable commercial importance and utility. Substances identified as upregulators of the utrophin promoter represent an advance in the fight against muscular dystrophy since they provide basis for design and investigation of therapeutics for

in vivo use.

In one aspect, the present invention provides an isolated nucleic acid comprising a promoter, the promoter comprising a sequence of nucleotides shown in Figure 1 or Figure 2. The promoter may comprise one or more fragments of the sequence shown in Figure 1 of Figure 2 sufficient to promote gene expression. The promoter may comprise or consist essentially of a sequence of nucleotides 5' to position 1440 in Figure 1 (human) or position 1183 in Figure 2 (mouse). Preferably the promoter comprises or consists essentially of nucleotides 1199 to 1440 of the human sequence shown in Figure 1, or the equivalent sequence in mouse, e.g. nucleotides 959 to 1183 of Figure 2.

An even smaller portion of this part of the sequences shown in Figure 1 of Figure 2 may be used as long as promoter activity is retained. Restriction enzymes or nucleases may be used to digest the nucleic acid, followed by an appropriate assay (for example as illustrated herein using luciferase constructs) to determine the minimal sequence required. A preferred embodiment of the present invention provides a nucleic acid isolate with the minimal nucleotide sequence shown in Figure 1 or Figure 2 required for promoter activity. The minimal promoter element is situated between the PvuII restriction site at position 1199 in the human sequence and the transcription start site at 1440 bp in the human sequence and between nucleotides 959 to 1183 in the mouse sequence (see Figure 2).

In one embodiment a promoter according to the present invention comprises or consists of sequence that is shown in Figure 3 to be conserved between the human and mouse sequences, e.g. the 25 nucleotide sequence: ACAGGACATCCCAGTGTGCAGTTCG spanning the transcriptional start site.

The promoter may comprise one or more sequence motifs or elements conferring developmental and/or tissue-specific regulatory control of expression. For instance, the promoter may comprise a sequence for muscle-specific expression, e.g. an E-box element/myoD binding site, such as CANNTG, preferably CAGGTG.

Other regulatory sequences may be included, for instance as identified by mutation or digest assay in an appropriate expression system or by sequence comparison with available information, e.g. using a computer to search on-line databases.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter.

The present invention extends to a promoter which has a nucleotide sequence which is allele, mutant, variant or derivative, by way of nucleotide addition, insertion, substitution or deletion of a promoter sequence as provided herein. Systematic or random mutagenesis of nucleic acid to make an alteration to the nucleotide sequence may be performed using any technique known to those skilled in the art. One or more alterations to a promoter sequence according to the present invention may increase or decrease promoter activity, or increase or decrease the magnitude of the effect of a substance able to modulate the promoter activity.

"Promoter activity" is used to refer to ability to initiate transcription. The level of promoter activity is quantifiable for instance by assessment of the amount of mRNA produced by transcription from the promoter or by assessment of the amount of protein product produced by translation of mRNA produced by transcription from the promoter. The amount of a specific mRNA present in an expression system may be determined for example using specific oligonucleotides which are able to hybridise with the mRNA and which are labelled or may be used in a specific amplification reaction such as the polymerase chain reaction. Use of a reporter gene as discussed further below facilitates determination of promoter activity by reference to protein production.

In various embodiments of the present invention a promoter which has a sequence that is a fragment, mutant, allele, derivative or variant, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the sequence of either the human or the mouse promoters shown in Figures 1 and 2, respectively, has at least about 60% homology with one or both of the shown sequences, preferably at least about 70% homology, more preferably at least about 80% homology, more preferably at least about 90% homology, more preferably at least about 95% homology. The sequence in accordance with an embodiment of the invention may hybridise with one or both of the shown sequences, or the complementary sequences (since DNA is generally double-stranded).

Similarity or homology (the terms are used interchangeably) or identity is preferably determined using GAP, from version 20 of GCG. This uses the algorithm of Needleman and Wunsch to align sequences inserting gaps as appropriate to improve the agreement between the two sequences. Parameters employed are the default ones: for nucleotide sequences - Gap Weight 50, Length Weight 3, Average Match 10.000, Average Mismatch 0.000; for peptide sequences - Gap Weight 8, Length Weight 2, Average

Match 2.912, Average Mismatch -2.003. Peptide similarity scores are taken from the BLOSUM62 matrix. Also useful is the TBLASTN program, of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-10, or BestFit, which is part of the Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). Sequence comparisons may be made using FASTA and FASTP (see Pearson & Lipman, 1988. Methods in Enzymology 183: 63-98). Parameters are preferably set, using the default matrix, as follows: Gapopen (penalty for the first residue in a gap): -12 for proteins / -16 for DNA; Gapext (penalty for additional residues in a gap): -2 for proteins / -4 for DNA; KTUP word length: 2 for proteins / 6 for DNA.

Nucleic acid sequence homology may be determined by means of selective hybridisation between molecules under stringent conditions.

Preliminary experiments may be performed by hybridising under low stringency conditions. For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which can be investigated further.

For example, hybridizations may be performed, according to the method of Sambrook et al. (below) using a hybridization solution comprising: 5X SSC (wherein `SSC' = 0.15 M sodium chloride; 0.15 M sodium citrate; pH 7), 5X Denhardt's reagent, 0.5-1.0% SDS, 100 μg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes - 1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65°C in 1X SSC and 1% SDS, changing the solution

every 30 minutes.

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology is (Sambrook et al., 1989): T_m = 81.5°C + 16.6Log [Na+] + 0.41 (% G+C) - 0.63 (% formamide) - 600/#bp in duplex.

As an illustration of the above formula, using [Na+] = [0.368] and 50-% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C. Such a sequence would be considered substantially homologous to the nucleic acid sequence of the present invention.

It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain. Other suitable conditions include, e.g. for detection of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

In a further embodiment, hybridisation of nucleic acid molecule to an allele or variant may be determined or identified indirectly, e.g. using a nucleic acid amplification reaction, particularly the polymerase chain reaction (PCR). PCR requires the use of two primers to specifically amplify target nucleic acid, so preferably two nucleic acid molecules

with sequences characteristic of the utrophin promoter are employed. Using RACE PCR, only one such primer may be needed (see "PCR protocols; A Guide to Methods and Applications", Eds. Innis *et al*, *Academic Press, New York*, (1990)).

Thus a method involving use of PCR in obtaining nucleic acid according to the present invention may include:

(a) providing a preparation of nucleic acid, e.g. from a muscle cell;

(b) providing a pair of nucleic acid molecule primers useful in (i.e. suitable for) PCR, at least one of said primers being a primer specific for nucleic acid according to the present invention;

(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR;

(d) performing PCR and determining the presence or absence of an amplified PCR product.

The presence of an amplified PCR product may indicate identification of an allele or other variant. The sequence may have the ability to promote transcription (i.e. have "promoter activity") in muscle cells, e.g. human muscle cells, or muscle-specific transcription.

Further provided by the present invention is a nucleic acid construct comprising a utrophin promoter region or a fragment, mutant, allele, derivative or variant thereof able to promoter transcription, operably linked to a heterologous gene, e.g. a coding sequence. By "heterologous" is meant a gene other than utrophin. Modified forms of utrophin are generally excluded. Generally, the gene may be transcribed into mRNA which may be translated into a peptide or polypeptide product which may be detected and preferably quantitated following expression. A gene whose encoded product may be assayed following expression is termed a "reporter gene", i.e. a gene which "reports" on promoter activity.

The reporter gene preferably encodes an enzyme which catalyses a reaction which produces a detectable signal, preferably a visually detectable signal, such as a coloured product. Many examples are known, including β-galactosidase and luciferase. β-galactosidase activity may be assayed by production of blue colour on substrate, the assay being by eye or by use of a spectrophotometer to measure absorbance. Fluorescence, for example that produced as a result of luciferase activity, may be quantitated using a spectrophotometer. Radioactive assays may be used, for instance using chloramphenicol acetyltransferase, which may also be used in non-radioactive assays. The presence and/or amount of gene product resulting from expression from the reporter gene may be determined using a molecule able to bind the product, such as an antibody or fragment thereof. The binding molecule may be labelled directly or indirectly using any standard technique.

Those skilled in the art are well aware of a multitude of possible reporter genes and assay techniques which may be used to determine gene activity. Any suitable reporter/assay may be used and it should be appreciated that no particular choice is essential to or a limitation of the present invention.

Expression of a reporter gene from the promoter may be in an *in vitro* expression system or may be intracellular (*in vivo*). Expression generally requires the presence, in addition to the promoter which initiates transcription, a translational initiation region and transcriptional and translational termination regions. One or more introns may be present in the gene, along with mRNA processing signals (e.g. splice sites).

Systems for cloning and expression of a polypeptide are discussed further below.

The present invention also provides a nucleic acid vector

comprising a promoter as disclosed herein. Such a vector may comprise a suitably positioned restriction site or other means for insertion into the vector of a sequence heterologous to the promoter to be operably linked thereto.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Procedures for introducing DNA into cells depend on the host used, but are well known.

Thus, a further aspect of the present invention provides a host cell containing a nucleic acid construct comprising a promoter element, as disclosed herein, operably linked to a heterologous gene. A still further aspect provides a method comprising introducing such a construct into a host cell. The introduction may employ any available technique, including, for eukaryotic cells, calcium phosphate transfection, DEAE-Dextran transfection, electroporation, liposome-mediated transfection and transduction using retrovirus.

The introduction may be followed by causing or allowing expression of the heterologous gene under the control of the promoter, e.g. by culturing host cells under conditions for expression of the gene.

In one embodiment, the construct comprising promoter and gene is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion in the construct of sequences which promote recombination with the genome, in accordance with standard techniques.

Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Current Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1994, the disclosure of which is incorporated herein by reference.

Nucleic acid molecules, constructs and vectors according to the present invention may be provided isolated and/or purified (i.e. from their natural environment), in substantially pure or homogeneous form, free or substantially free of a utrophin coding sequence, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the promoter sequence. Nucleic acid according to the present invention may be wholly or partially synthetic. The term "isolate" encompasses all these possibilities.

Nucleic acid constructs comprising a promoter (as disclosed herein) and a heterologous gene (reporter) may be employed in screening for a substance able to modulate utrophin promoter activity. For therapeutic purposes, e.g. for treatment of muscular dystrophy, a substance able to up-regulate expression of the promoter may be sought. A method of screening for ability of a substance to modulate activity of a utrophin promoter may comprise contacting an expression system, such as a host cell, containing a nucleic acid construct as herein disclosed with a test or candidate substance and determining expression of the heterologous gene. The level of transcription of the heterologous gene, or the level of heterologous protein may be determined. The level of protein may be determined by measuring the amount of protein, or the activity of the protein, using techniques known to those skilled in the art.

Alternatively, or additionally a method of screening for

ability of a substance to modulate activity of a utrophin promoter may comprise contacting a cell containing an endogenous utrophin gene (e.g. a mammalian muscle cell) with a test substance and measuring the level of RNA transcription or protein expression using binding members specific for the nucleic acid or polypeptides disclosed herein. Specific binding members include antibodies and nucleic acid probes.

The level of expression in the presence of the test substance may be compared with the level of expression in the absence of the test substance. A difference in expression in the presence of the test substance indicates ability of the substance to modulate gene expression. An increase in expression of the heterologous gene compared with expression of another gene not linked to a promoter as disclosed herein indicates specificity of the substance for modulation of the utrophin promoter.

A promoter construct may be transfected into a cell line using any technique previously described to produce a stable cell line containing the reporter construct integrated into the genome. The cells may be grown and incubated with test compounds for varying times. The cells may be grown in 96 well plates to facilitate the analysis of large numbers of compounds. The cells may then be washed and the reporter gene expression analysed. For some reporters, such as luciferase, the cells will be lysed then analysed. Previous experiments testing the effects of glucocorticoids on the endogenous utrophin protein and RNA levels in myoblasts have already been described [12,13] and techniques used for those experiments may similarly be employed.

Constructs comprising one or more developmental and/or time-specific regulatory motifs (as discussed) may be used to screen for a substance able to modulate the corresponding aspect of the promoter activity, e.g. muscle-specific

expression.

Following identification of a substance which modulates or affects utrophin promoter activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

As noted above, the inventors also identified a novel coding sequence (Exon IB) which encodes a novel utrophin N-terminus.

According to a further aspect of the present invention there is provided a nucleic acid molecule which has a nucleotide sequence encoding a polypeptide which includes the amino acid sequence shown in Figure 1 or Figure 2.
Such a polypeptide may include other utrophin sequences, and the nucleic acid molecule may be in the form of a utrophin "mini-gene"(discussed further below).

Such a polypeptide may include non-utrophin (i.e. heterologous or foreign) sequences and thereby form a larger fusion protein. For example, such a fusion protein could be used to target a non-utrophin polypeptide to muscle membranes.

The coding sequence included may be that shown in Figure 1 or Figure 2 or it may be a mutant, variant, derivative or allele of the sequence shown. The sequence may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may

include a sequence different from the sequences shown in Figure 1 or Figure 2 yet encode a polypeptide with the same amino acid sequence. The amino acid sequences shown in Figure 1 and figure 2 consist of 31 residues.

On the other hand the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequences shown in Figure 1 or Figure 2. Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant, derivative or allele of the sequences shown in Figure 1 and Figure 2 are further provided by the present invention. Nucleic acid encoding such a polypeptide may show at the nucleotide sequence and/or encoded amino acid level greater than about 60% homology with the coding sequence and/or the amino acid sequence shown in Figure 1 or Figure 2, greater than about 70% homology, greater than about 80% homology, greater than about 90% homology or greater than about 95% homology. Determination of homology is discussed elsewhere herein.

A polypeptide which is a variant, allele, derivative or mutant may have an amino acid sequence which differs from that given in a figure herein by one or more of addition, substitution, deletion and insertion of one or more amino acids. Preferred such polypeptides have wild-type function, that is to say have one or more of the following properties: immunological cross-reactivity with an antibody reactive the polypeptide for which the sequence is given in Figure 1 or Figure 2; sharing an epitope with the polypeptide for which the amino acid sequence is shown in Figure 1 or Figure 2(as determined for example by immunological cross-reactivity between the two polypeptides); a biological activity which is inhibited by an antibody raised against the polypeptide whose sequence is shown in Figure 1 or Figure 2; ability to bind muscle membrane, ability to bind actin; ability to bind DPC.

Variations in amino acid sequence include "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Particular amino acid sequence variants may differ from that shown in Figure 1 or Figure 2 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, or 5-10 amino acids.

According to one aspect of the present invention there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding a polypeptide with utrophin function. Utrophin nucleotide sequences which may be included in the nucleic acid molecule are disclosed in WO 97/922696 which is incorporated herein by reference.

See also Figure 8 and Figure 9 for disclosure of nucleic acid molecules and polypeptides according to the present invention, comprising the exon IB sequence of the invention.

A polypeptide with utrophin function is able to bind actin and able to bind the dystrophin protein complex (DPC).

The nucleic acid molecule may be an isolate, or in an isolated and/or purified form, that is to say not in an environment in which it is found in nature, removed from its natural environment. It may be free from other nucleic acid obtainable from the same species, e.g. encoding another polypeptide.

In one embodiment, nucleic acid molecule is a "mini-gene", i.e. the polypeptide encoded does not correspond to full-length utrophin but is rather shorter, a truncated version (Utrophin mini-genes are discussed in WO97/22696). For instance, part or all of the rod domain may be missing, such

that the polypeptide comprises an actin-binding domain and a DPC-binding domain but is shorter than naturally occurring utrophin. In a full-length utrophin gene including what are identified herein as exons 1A and 1B, the actin-binding domain is encoded by nucleotides 1-739, while the DPC-binding domain (CRCT) is encoded by nucleotides 8499-10301 (where 1 represents the start of translation). See also Figure 8. The respective domains in the polypeptide encoded by a mini-gene according to the invention may comprise amino acids corresponding to those encoded by these nucleotides in the full-length coding sequence. In one embodiment, a minigene according to the present invention comprises or consists of the amino acid sequence encoded by nucleotides 1-739 and 8499-10301 of the A isoform of utrophin in which exon 1B as identified herein is substituted for exons 1A and 2A. The sequence of such a minigene can be constructed by the ordinary skilled person using information disclosed herein, taking into account the content of WO97/22696 and Tinsley et al, *Nature* (1996) 384:349. The nucleic acid sequence and predicted amino acid sequence encoded by a `mini-gene' according to the present invention are shown in Figure 9.

Advantages of a mini-gene over a sequence encoding a full-length utrophin molecule or derivative thereof include easier manipulation and inclusion in vectors, such as adenoviral and retroviral vectors for delivery and expression.

A further preferred non-naturally occurring nucleic acid molecule encoding a polypeptide with the specified characteristics is a chimaeric construct wherein the encoding sequence comprises a sequence obtainable from one mammal, preferably human ("a human sequence"), and a sequence obtainable from another mammal, preferably mouse ("a mouse sequence"). Such a chimaeric construct may of course comprise the addition, insertion, substitution and/or deletion of one or more nucleotides with respect to the parent mammalian

sequences from which it is derived. Preferably, the part of the coding sequence which encodes the actin-binding domain comprises a sequence of nucleotides obtainable from the mouse, or other non-human mammal, or a sequence of nucleotides derived from a sequence obtainable from the mouse, or other non-human mammal.

In a preferred embodiment, the sequence of nucleotides encoding the polypeptide comprises sequence GAGGCAC at residues 331-337 and/or the sequence GATTGTGGATGAAAACAGTGGG at residues 1453-1475 (using the conventional numbering from the initiation codon ATG), and a sequence obtainable from a human.

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridise with one or more fragments of a nucleic acid sequence shown in Figure 1 or Figure 2 particularly fragments of relatively rare sequence, based on codon usage or statistical analysis. The amino acid sequence information provided may be used in design of degenerate probes/primers or "long" probes. A primer designed to hybridise with a fragment of the nucleic acid sequence shown may be used in conjunction with one or more oligonucleotides designed to hybridise to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE" (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an oligonucleotide linker and PCR is performed using a primer which hybridises with the sequence shown in the figures and a primer which hybridises to the oligonucleotide linker.

Nucleic acid isolated and/or purified from one or more cells (e.g. human, mouse) or a nucleic acid library derived from nucleic acid isolated and/or purified from cells (e.g. a cDNA library derived from mRNA isolated from the cells), may be probed under conditions for selective hybridisation and/or subjected to a specific nucleic acid amplification reaction

such as the polymerase chain reaction (PCR).

A method may include hybridisation of one or more (e.g. two) probes or primers to target nucleic acid. Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. A screening procedure, chosen from the many available to those skilled in the art, is used to identify successful hybridisation events and isolated hybridised nucleic acid.

Probing may employ the standard Southern blotting technique. For instance DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells.

Preliminary experiments may be performed by hybridising under low stringency conditions various probes to Southern blots of DNA digested with restriction enzymes. Suitable conditions would be achieved when a large number of hybridising fragments were obtained while the background hybridisation was low. Using these conditions nucleic acid libraries, e.g. cDNA libraries representative of expressed sequences, may be searched.

It may be necessary for one or more gene fragments to be ligated to generate a full-length coding sequence. Also, where a full-length encoding nucleic acid molecule has not been obtained, a smaller molecule representing part of the

full molecule, may be used to obtain full-length clones. Inserts may be prepared from partial cDNA clones and used to screen cDNA libraries.

Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on. Exemplary conditions have been discussed already above.

Nucleic acid according to the present invention may form part of a cloning vector and/or a vector from which the encoded polypeptide may be expressed. Polypeptide expression is discussed below. Suitable vectors can be chosen or constructed, containing appropriate and appropriately positioned regulatory sequences, as discussed elsewhere herein.

A further aspect of the present invention provides a polypeptide which comprises the amino acid sequence shown in Figure 1 or Figure 2. As mentioned earlier such a polypeptide may include other utrophin sequences or may include heterologous sequences.

Polypeptides which are amino acid sequence variants, alleles, derivatives or mutants are also provided by the present invention. Such polypeptides are discussed elsewhere herein.

The skilled person can use the techniques described herein and others well known in the art to produce large amounts of peptides, for instance by expression from encoding nucleic acid.

In a further aspect the invention provides a method of making a polypeptide, the method including expression from nucleic

acid encoding the polypeptide (generally nucleic acid according to the invention). This may be conveniently be achieved by growing in culture a host cell containing such a vector, under suitable conditions which cause or allow expression of the polypeptide. Polypeptides may also be expressed in in vitro systems such as reticulocyte lysate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. A common, preferred bacterial host is *E. coli*.

Thus, a further aspect of the present invention provides a host cell containing heterologous nucleic acid encoding a polypeptide as disclosed herein.

The nucleic acid may be integrated into the genome (e.g. chromosome) of the host cell or may be on an extra-chromosomal vector within the cell, or otherwise identifiably heterologous or foreign to the cell.

A still further aspect provides a method comprising introducing such nucleic acid into a host cell. Suitable techniques are discussed elsewhere herein.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.
The polypeptide encoded by the nucleic acid may be expressed from the nucleic acid *in vitro*, e.g. in a cell-free system or in cultured cells, or *in vivo*.
If the polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into

the culture medium.

Peptides can also be generated wholly or partly by chemical synthesis. The compounds of the present invention can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

The present invention also includes active portions, fragments, derivatives and functional mimetics of the polypeptides of the invention. An "active portion" of a polypeptide means a peptide which is less than said full length polypeptide, but which retains a biological activity, such as a biological activity selected from binding to ligand, binding to muscle membrane. Such an active fragment may be included as part of a fusion protein, e.g. including a polypeptide which is to be targetted to the muscle membrane.

A "fragment" of a polypeptide generally means a stretch of amino acid residues of about five to twenty-five contiguous amino acids, typically about ten to twenty contiguous amino acids. Fragments of the novel N-terminus polypeptide sequence may include antigenic determinants or epitopes useful for

raising antibodies to a portion of the amino acid sequence, or may be sequence useful for targetting to muscle membrane. Alanine scans are commonly used to find and refine peptide motifs within polypeptides, this involving the systematic replacement of each residue in turn with the amino acid alanine, followed by an assessment of biological activity.

Preferred fragments of exon IB polypeptide include those comprising or consisting of an epitope which may be used for instance in raising or isolating antibodies. Variant and derivative peptides, peptides which have an amino acid sequence which differs from one of these sequences by way of addition, insertion, deletion or substitution of one or more amino acids are also provided by the present invention.

A "derivative" of a polypeptide or a fragment thereof may include a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve one or more of insertion, addition, deletion or substitution of one or more amino acids, which may be without fundamentally altering the qualitative nature of biological activity of the wild type polypeptide. Also encompassed within the scope of the present invention are functional mimetics of active fragments of the exon 1B polypeptides provided (including alleles, mutants, derivatives and variants). The term "functional mimetic" means a substance which may not contain an active portion of the relevant amino acid sequence, and probably is not a peptide at all, but which retains in qualitative terms biological activity of natural exon 1B polypeptide. The design and screening of candidate mimetics is described in detail below.

A polypeptide according to the present invention may be isolated and/or purified (e.g. using an antibody) for instance

after production by expression from encoding nucleic acid (for which see below). Thus, a polypeptide may be provided free or substantially free from contaminants with which it is naturally associated (if it is a naturally-occurring polypeptide). A polypeptide may be provided free or substantially free of other polypeptides. Polypeptides according to the present invention may be generated wholly or partly by chemical synthesis. The isolated and/or purified polypeptide may be used in formulation of a composition, which may include at least one additional component, for example a pharmaceutical composition including a pharmaceutically acceptable excipient, vehicle or carrier. A composition including a polypeptide according to the invention may be used in prophylactic and/or therapeutic treatment as discussed below.

A polypeptide, peptide, allele, mutant, derivative or variant according to the present invention may be used as an immunogen or otherwise in obtaining specific antibodies. Antibodies are useful in purification and other manipulation of polypeptides and peptides, diagnostic screening and therapeutic contexts.

Accordingly, a further aspect of the present invention provides an antibody able to bind specifically to the polypeptide whose sequence is given in Figure 1 or Figure 2. Such an antibody may be specific in the sense of being able to distinguish between the polypeptide it is able to bind and other human (or mouse) polypeptides for which it has no or substantially no binding affinity (e.g. a binding affinity of about 1000x less). Specific antibodies bind an epitope on the molecule which is either not present or is not accessible on other molecules. Antibodies according to the present invention may be specific for the wild-type polypeptide. Antibodies according to the invention may be specific for a particular mutant, variant, allele or derivative polypeptide as between that molecule and the wild-type polypeptide, so as

to be useful in diagnostic and prognostic methods as discussed below. Antibodies are also useful in purifying the polypeptide or polypeptides to which they bind, e.g. following production by recombinant expression from encoding nucleic acid.

Preferred antibodies according to the invention are isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention.

Antibodies may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al., 1992, Nature 357: 80-82). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained

from an organism which has been exposed to the antigen of interest.

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic molecules and molecules whose shape mimicks that of an antibody enabling it to bind an antigen or epitope.

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')2 fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other changes. It will further be understood by those skilled in the art that a monoclonal antibody can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP184187A, GB 2188638A or EP-A-0239400. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

The reactivities of antibodies on a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody with an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions

between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge. Particular embodiments of antibodies according to the present invention include antibodies able to bind and/or which bind specifically, e.g. with an affinity of at least 10^{-7} M, to the peptides shown in Figure 1 or Figure 2.

Antibodies according to the present invention may be used in screening for the presence of a polypeptide, for example in a test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid therefor.

An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling molecules, buffer solutions, elutants and so on. Reagents may be provided within containers which protect them from the external environment, such as a sealed vial.

The present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of utrophin promoter activity, or to a polypeptide, or nucleic acid molecule in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a

method comprising administration of such a composition to a patient, e.g. for increasing utrophin expression for instance in treatment of muscular dystrophy, use of such a substance in manufacture of a composition for administration, e.g. for increasing utrophin expression for instance in treatment of muscular dystrophy, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

Administration will preferably be in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, eg decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

Pharmaceutical compositions for oral administration may be in

tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Instead of a substance identified using a promoter as disclosed herein, a mimetic or mimick or the substance may be designed for pharmaceutical use. The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, eg peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing may be used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property.

Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, eg by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled to according its physical properties, eg stereochemistry, bonding, size and/or charge, using data from a range of sources, eg spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process. In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Mimetics of substances identified as having ability to

modulate utrophin promoter activity using a screening method as disclosed herein are included within the scope of the present invention.

Modifications to and further aspects and embodiments of the present invention will be apparent to those skilled in the art. All documents mentioned herein are incorporated by reference.

Experimental basis for and embodiments of the present invention will now be described in more detail, by way of example and not limitation, and with reference to the following figures:

Figure 1 shows the sequence of the human exon 1B and promoter B. Numbering corresponds to the insert of pBSX2.0. The deduced translation of exon 1B is shown. The positions of features such as restriction sites, IL-6 response element and Alu repetitive elements are shown.

Figure 2 shows the sequence of the mouse exon 1B and promoter B. Numbering corresponds to the insert of pBSX8.0. The deduced translation of exon 1B is shown. The positions of features such as restriction sites, IL-6 response element and Alu repetitive elements are shown.

Figure 3 shows the sequence alignment of human (top) and mouse (bottom) exon 1B (in upper case) and promoter B. Numbering corresponds to the inserts of pBSX2.0 and pBSX8.0, respectively. The human PvuII site (see Figure 7) is indicated. The open triangle indicates the position at which the luciferase coding sequence was inserted to make pGL3/UtroB/F (see below). The deduced translation of exon 1B is shown; amino acids marked in bold type are identical between the human and mouse sequences. The conserved splice donor consensus is shown in grey. Two putative Ap1 sites and

an initiator-like element (Inr) are 100% conserved and indicated in black. A solid arrow marks the single transcription start indicated by primer extension; figures adjacent to the sequence indicate the number of individual 5'RACE clones that terminated at the positions shown.

Figure 4 shows the position of the primers used in RT-PCR of exon 1B-containing utrophin transcript, and the probes used to probe the PCR products. Primers specific to exon 1B (BF31) and utrophin C-terminus (CT2) were used to amplify 9816bp of utrophin cDNA. The products were blotted and probed with U41, U107, BR4 and U16 as indicated. The diagram is not to scale; numbering refers to the nucleotide sequence of the full-length cDNA. The corresponding functional domains of the protein are indicated above: actin binding domain; rod, rod domain; Cys, cysteine rich domain, C-Term; C-terminal domain.

Figure 5 shows a schematic representation of (A) human YAC and (B) mouse PAC contigs showing position of exons within the genomic map. Key to mouse restriction sites: C, ClaI; S, SacII; B, BssHII; X, XhoI. (C) shows the nomenclature for utrophin promoters, exons and transcripts.

Figure 6 shows the *in vitro* activity of utrophin promoter B. (A) shows normalised luciferase activity following transfection of three different human cell types with either pGL3/utroB/F (`forward construct') or pGL3/utroB/R (`reverse construct').

Figure 7 shows deletion analysis of promoter B. The 1.5kb insert of pGL3/utroB/F was deleted at its 5' and 3' ends using the internal restriction sites indicated. Reporter activity was assayed following transient transfection of IN157 and CL11T47 cells.

Figure 8 shows conceptual translation of exon 1B as part of

utrophin, showing a nucleotide sequence and encoded polypeptide according to embodiments of the present invention.

Figure 9 shows the nucleic acid and predicted amino acid sequence of a utrophin B isoform `minigene'.

Figure 10 shows the dosage dependence of IL-6 mediated expression from the isoform B promoter.

Oligonucleotides, PCR, RT-PCR and 5'RACE

PCR and RT-PCR were performed as described (Blake, *et al.* (1996) *J Biol Chem* **271**, 7802-7810). Oligonucleotide sequences (5' to 3') were:

UM83	gatgttcctg tgaggccttc gag,
UM82	cactcttgga aaatcgagcg t,
U16	actatgatgt ctgccagagt tg,
U107	gatccaatag cttccttcca tcttt,
UBF	tggaaaaagt ggaggttgga,
BR2	tccaacctcc actttttcca,
BR4	gcctggagag ctacatgccc t,
BF8	ctccacatct ttttcctcat catct,
BF9	gattgtggtg atggttgtag aa,
BR10	gattgtggtg atggttgtag aa,
BR14	gatgatgagg aaaaagatgt ggag,
BF15	aaacccaaaa taacacagga catc,
BF16	agtgtaactt ctctctggtg,
BF31	taagcagatg taggtgatga gc,
BF42	gctgcttttg ttgtccactt c,
BR43	atagcttcct tccatctttg ag,
CT2	ctccacgttc ttccctctct act,
2ApF	gcgtgcagtg gaccattttt cagattta,
1BpF	cgctgcagca gccaccacat ttcgttg,
3pR	gcgtgcagat cgagcgttta tccatttg.

5' RACE was undertaken using adapter-ligated mouse heart cDNA (Marathon-Ready, Clontech), following the manufacturer's

protocol, using the supplied adapter primers with nested mouse utrophin primers UM83 (exon 4) and UM82 (exon 3). Products were cloned in pGEM-T (Promega). Human exon 1B was isolated from skeletal muscle cDNA by PCR using mouse primers UBF and UM83. 5'RACE was used to clone the 5' end of human exon 1B, using primers U107 and BR4. Full-length utrophin RT-PCR was done as described (Blake, *et al.* (1996) *J Biol Chem* **271**, 7802-7810.), but using Boehringer Expand Reverse Transcriptase and Long Template PCR reagents, and a primer annealing temperature of 59°C. Semi-quantitative RT-PCR was performed using primers BF42 and BR43 to amplify utrophin B, and commercial primers (Stratagene) to amplify glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Exponential amplification was established by withdrawing samples from thermal cycling at 1 cycle intervals over a range of 5 cycles, predicted to span the exponential range following initial experiments in which samples were withdrawn at 5 cycle intervals. Products were blotted and probed with labelled BR4 or a 600bp GA3PH probe. Band intensities were quantified using a Storm phosphoimager. A graph of $\log_2$[band intensity] versus cycle number showed a linear relationship with gradient $\simeq$ 1, indicating near-perfect exponential amplification. The band intensities at any given cycle over this range are therefore directly proportional to the amount of cDNA in the original samples.

Genomic Mapping and Clones

Human YACs are as previously described (Pearce, *et al.* (1993) *Hum Mol Genet* **2**, 1765-72). Southern blots of restriction digested YAC DNA were probed with end-labelled BR4. A 3.0kb hybridising XbaI fragment was cloned from YAC 4X124H10 (a YAC clone which contains a human genomic DNA insert) into pBlueScript (Stratagene) generating pBSX2.0. Mouse PACs were identified from the RPCI21 library. A 398bp exon 1B/promoter B DNA probe (UB400) encompassing human positions 1129 to 1527 was used for exon 1B mapping. Library filters were screened with probes to exons 1A-5 (Dennis, *et al.* (1996) *Nucleic Acid*

Res **24**, 1646-52) and UB400. Eleven PACs were identified, and four of these arranged into a contig by restriction mapping. An 8.0kb XbaI fragment from PAC 110C24, that hybridised with UB400, was cloned in pBlueScript generating pBSX8.0.

Northern Blots and Probes

A human multiple tissue northern blot and b-actin control cDNA probe were obtained from Clontech. A utrophin C-terminal cDNA probe, encompassing the last 4.0kb of the utrophin message, was generated by PCR. Human exon 1B sequence between positions 1480 and 1596 was cloned into pGEM-T and an exon 1B antisense riboprobe was transcribed (In Vitro Transcription Kit, Promega) from the SP6 promoter following linearisation of the plasmid with NcoI. Hybridisation was carried out at 70°C in 50% formamide hybridisation buffer (Ausubel, *et al.* (1999) *Current Protocols in Molecular Biology* (Wiley).) and the filter was washed at 75°C in 0.1xSSC, 0.1%SDS for 2 hours.

RNase Protection

Specific probes spanning the exon 1B/3 and exon 2A/3 boundaries were obtained by PCR amplification of mouse heart cDNA using primers 2ApF, 1BpF and 3pR. Products were cloned in the PstI site of pDP18 (Ambion) and sequenced. Plasmids were linearised with EcoR1 (1B) or BamH1 (2A); labelled antisense riboprobe was transcribed from the T7 promoter and gel purified. RNase protection was carried out using RPAIII kit (Ambion) following the manufacturer's instructions (30μg total RNA unless stated, hybridisation temperature 42°C, RNase A/T1 dilution 1:200). Following electrophoretic separation, band intensities were quantified as above, and corrected for the amount of label present in each protected fragment.

Promoter/Reporter Constructs

Reporter constructs were generated by PCR amplification of the human sequence between positions 39 and 1503, using pBSX2.0 as template. Pfu polymerase was used with primers BF9 and BR14.

Following 15 cycles of 96°C for 45 seconds, 62°C for 45 seconds, 72°C for 4 minutes, products were dA-tailed and cloned in pGEM-T. Clones were identified with product in both orientations and insert, liberated by digestion with SacI/NcoI, was cloned into the SacI/NcoI sites of a promoterless luciferase reporter plasmid (pGL3 basic, Promega), generating constructs with insert in forward (pGL3/utroB/F) and reverse (pGL3/UtroB/R) orientation with respect to the coding sequence of luciferase. Deletions of the forward construct were generated by cleavage at SpeI, NdeI, EcoRI and PvuII sites in the insert, followed by religation to sites in the 5' or 3' polylinker. Constructs were sequenced completely.

Cell Culture and Transfections

Three human cell lines (IN157 rhabdomyosarcoma (Nielsen et al., 1993, *Mol Cell Endocrinol* 93: 87-95), CL11T47 kidney epithelial and HeLa cervical epithelial (*Cancer Research, 1952* 12: 264)were maintained as described (Dennis, *et al.* (1996) *Nucleic Acid Res* **24**, 1646-52). 2μg pGL3/utroB/F or R, or its molar equivalent, mixed with 0.5μg of LacZ control plasmid (pSV-β-gal, Promega) was transfected in each well of 6 well plates using Superfect (Qiagen), following the manufacturer's protocol. 48 hours later, cells were harvested and cell extracts were assayed for luciferase and β-galactosidase activity as described (Dennis, *et al.* (1996) *Nucleic Acids Res* **24**, 1646-52). Luciferase activity was standardised to β-galactosidase activity in each individual sample to control for transfection efficiency. Results are expressed as mean luciferase/β-galactosidase ratio for four individual transfections. Error bars indicate the standard error of the mean. For comparison of different constructs within the same cell line, results were standardised to those obtained with pGL3/utroB/F and are expressed as % of this value. For comparison of constructs between cell lines, results were standardised to those obtained with a luciferase-SV40

promoter/enhancer plasmid (pGL3 control, Promega) that generates high levels of reporter activity in all cell lines tested.

Primer Extension

Primer extension was carried out as described (18); end-labelled primer BR2 was annealed to 0, 30 or 50μg mouse heart total RNA at 58°C for 20 minutes, and extended at 42°C for 40 minutes. Products were separated on a 6% polyacrylamide gel, under denaturing conditions, alongside a sequencing ladder generated from pBSX8.0 using primer BR2.

Results

An alternative 5' exon in utrophin mRNA

Utrophin from a mouse heart cDNA library was amplified by 5'RACE, and the resulting products cloned and sequenced. Of 12 clones, 8 contained novel sequence 5' of exon 3. Below, we present evidence that the novel sequence is a single alternative 5' exon of utrophin containing a translational initiation codon. We refer to this sequence as `exon 1B' to distinguish it from the previously described 5' cDNA sequence comprising untranslated exon 1A and exon 2A which contains the translational start (Figure 5c).

Figure 3 shows a sequence comparison of human and mouse exon 1B, and genomic flanking sequence. The position and phase of the splice junction at the 5' end of exon 3 is identical for both exon 1B- and exon 2A- containing transcripts. Exon 1B contains a putative ATG translation initiation codon and open reading frame, in-frame with that of exon 3, predicting a novel 31 amino acid N-terminus to the utrophin protein. The context of the ATG codon is predicted to be favourable for translation in that there is a purine at position -3 (bold in Figure. 3) (33). Human and mouse exons 1B show 82% nucleotide identity. The predicted translations are 84% identical and 94%

similar. The position and context of the ATG codon are conserved. The human sequence contains a second putative ATG codon immediately 5' (position 1511, solid bar in Figure.1), followed by a TAG stop codon. As this ATG does not adhere to the Kozak consensus, is not associated with an open reading frame and is not present in the mouse sequence, we predict that this is not a functional translation start. A similar feature is present in human exon 2A, where the 5'UTR contains a short open reading frame prior to the true translation start.

The transcript associated with exon 1B

A human multiple tissue northern blot was probed with an exon 1B anti-sense riboprobe. A single hybridising 13kb band was observed, identical to that produced by probing the same blot with a cDNA encompassing 4kb of the utrophin C-terminus, indicating that exon1B is exclusively associated with a full-length utrophin mRNA. Exon 1B is ubiquitously expressed, and appears most abundant in heart and pancreas, and least abundant in the brain, relative to β-actin. This is similar to the expression profile of total full-length utrophin.

RT-PCR was employed to confirm the association of exon 1B with a utrophin mRNA predicted to give rise to functional protein (Figure.4). Amplification of first strand cDNA from IN157 cells utilising a forward primer specific to exon 1B (BF3I) and a reverse primer within the utrophin C-terminus (CT2) produced a product of expected size. Successive hybridisation of this PCR product with domain-specific probes; U41, UBR4, U107 and U16, confirmed that exon 1B is associated with a utrophin transcript spanning the full coding sequence of the gene.

The expression profiles of exons 1B and 2A were examined using RNase protection. Specific riboprobes corresponding to the exon 1B/3 and 2A/3 boundaries were simultaneously hybridised with total RNA, allowing direct quantitation of transcript

abundance. B-utrophin is the most abundant form in the heart, whereas exon 2A-containing transcripts predominate in the kidney. Approximately equal amounts of exons 1B and 2A were observed in the brain and in skeletal muscle.

Mapping and cloning of genomic sequence associated with exon 1B

Using probe BR4, exon 1B was mapped within our previously described human YAC contig (26) encompassing the 5' end of the utrophin locus (Figure.5a). A hybridising band was seen with YAC 4X124H10 but not 4X23E3 or 5C2 indicating that exon 1B lies within the 120kb intron 2 of the utrophin gene. A subsequent database search identified a clone from the HGMP human chromosome 6 sequencing project, containing exons 1A, 2A and 1B. This indicated that exon 1B lies 52.2kb 3' of exon 2A (Figure.5a). Probing the mouse genomic PAC library (RPCI21 from P. DeJong, Roswell Park Cancer Institute) with utrophin exons 1A, 1B and 2- 5 inclusive identified a series of genomic PACs spanning the 5' end of the mouse utrophin gene. Four of these PACs were assembled into a contig of the region. Hybridisation with UB400 confirmed that exon 1B lies within intron 2 in the mouse (Figure.5b), approximately 50kb 3' of exon 2.

Human and mouse genomic fragments were obtained from the YAC and PAC libraries, respectively. Genomic sequence encompassing exon 1B was obtained by an Xba I digest of YAC 4X124H10 (human 3kb fragment)and PAC110c24 (mouse 8.8kb fragment). These fragments were sub-cloned into pBluescript vector, the human fragment was deleted to 2kb during the sub-cloning. The plasmid clones were designated pBSX2.0 (human) and pBSX8.0 (mouse). Comparison of the cDNA and genomic sequence showed no evidence of a further 5' exon in the transcript associated with exon 1B, suggesting that the genomic flanking sequence contained the transcription start and promoter element responsible for exon 1B expression. Our

nomenclature for utrophin 5' exons, transcripts and promoters appears in Figure 5c.

Promoter B

1.5kb of human genomic sequence 5' of exon 1B, including the 5'UTR of exon 1B, was cloned in both orientations into a promoterless luciferase reporter vector. Three human cell lines (IN157 rhabdomyosarcoma, CL11T47 kidney epithelial and HeLa cervical epithelial) were transiently transfected with these constructs. These three lines were chosen because they are known to express utrophin mRNA and protein at different levels. Reporter activity was detected at significantly higher levels in cells transfected with the forward than the reverse orientation construct, indicating promoter activity (Figure 6). Interestingly, the level of activity varied between cell lines by an order of magnitude. Semi-quantitative RT-PCR demonstrated that the variation of luciferase expression mimicked the transcription profile of endogenous utrophin exon 1B. In contrast, the GA3PDH control showed identical amplification in all cDNA samples, indicating that the differences seen in B-utrophin amplification have arisen from differences in the level of expression of the endogenous B-utrophin transcript in these cells lines. These data show that the 1.5kb of genomic sequence 5' of exon 1B utilised in these reporter clones contains the necessary signals to initiate transcription of exon 1B, and regulatory elements that determine the level of expression in these cell lines.

To further delineate important elements within this region, a series of 5' and 3' deletions of promoter B were made, and the *in vitro* activity of each one assayed (Figure 7). A 300bp element, contained within clone pGL3/utroB/F/D5' Pvu 1199, retains 70% activity of the full 1.5kb construct in expressing cell lines, and shows 74% identity between human and mouse (Figure.3). Homology falls to 50% when sequence further 5' if the human PvuII site is compared with corresponding mouse

sequence using a 35bp window. Homology was determined using GAP, from version 20 of GCG, with default parameters as noted already above.

Promoter B transcription start site

The 5' ends of 8 human and 4 mouse 5'RACE clones clustered around a putative cap site in the genomic sequence (Figure.3). None of the 5'RACE clones generated by amplification across the exon 3/exon 1B boundary extended further upstream. RT-PCR was carried out using forward primers around this region with a reverse primer in exon 4. A product of expected size was amplified from IN157 cDNA by primers BF42 and BF8, but not BF16 or BF15, indicating that the transcription start is within the 18bp that separates the two primers BF15 and BF42. These 18 bases contain the putative cap site and the cluster of RACE clone 5' ends.

To map the start site accurately, primer extension using an exon 1B reverse primer and mouse heart RNA was employed. This yielded a single product, indicative of a single transcription start site. Transcription initiates at mouse position 1183 within a 25-bp motif, which is 100% conserved between human and mouse. Part of this motif, spanning the cap site, is a 6/7 base match for the initiator consensus, and correspondingly shows homology to the initiators of other genes. The transcription start site is homologous to the initiators of other promoters. Consensus 1, initiator consensus derived from sequence comparison of Inr^+ genes (Azizkhan, et al. (1993) *Critical Reviews in Eukaryotic Gene Expression* **3**, 229-254.); consensus 2, experimentally-derived consensus for functional initiator (Javahery, *et al.* (1994) *Molecular and Cellular Biology* **14**, 116-127.); TdT, terminal deoxynucleotidyl transferase; hRAR, human retinoic acid receptor a; mCREB, mouse cAMP response element binding protein. Transcribed sequence is indicated in bold uppercase. We consider this promoter to be of the $TATA^-Inr^+$ type.

Assaying for substances which modulate utrophin promoter activity

Method 1:

This method uses a mouse *mdx*-H2K myoblast line stably transfected with a human 7.0kb utrophin promoter-luciferase construct. On day 1 myoblast cells transfected with the construct are plated out in 6-well dishes, with compound or DMSO-only for the negative controls.

4 x 6 well plates are used for every 3 compounds (the compounds are dissolved in DMSO and stored prior to use). For example, compound A, or B, or C were each added to 1 well, while the remaining 3 wells contain only DMSO. This results in 4 wells containing each compound and 12 wells with DMSO alone. Due to the inherent noise of both the harvesting/assay and cell seeding/growth steps, this is the minimum number that results in meaningful analysis. Setting up the plates in this way means that the data really are paired, and can be analysed with a paired student T test. This provides a more powerful statistical analysis rather than putting each compound on a different plate and comparing it with a control plate.

On Day 4 the cells are harvested and luciferase quantitation and pairwise analysis is carried out.

Method 2:

Compounds which up-regulate the endogenous utrophin promoter are be found using *mdx*-H2K myoblasts that are not transfected with the utrophin promoter-luciferase construct. *Mdx*-myoblasts can be used to mimic utrophin transcprition and protein stability in dystrophin-deficient cells.

Identification of utrophin protein expression

Quantitative Western Blotting is used to measure the level of

utrophin expression (Tinsley JM, *et al.*, *Nature Medicine* **4**, 1441-1444.) Using 6 well plates and treating with compound as described above generates enough total protein sample to test by Western blotting. Antibodies specific to the A protein or B protein are used to quantify levels of either protein.

Identitification of utrophin RNA expression

Quantitative ribonuclease protection is used to analyse levels of utrophin expression. A pairwise design is used, as described above, but more cells are necessary. To see bands clearly, about 20-30μg total RNA is used. Each compound and control will need a 175 cm^2 tissue culture flask. A dual probe to simultaneously identify the A transcript and B transcript is be used.

Using the two techniques described compounds are identified after cell treatment which modulate utrophin levels. The same techniques are used for *in vivo* animal experiments where the compound is administered to dystrophin deficient *mdx* mice.

Interleukin-6 (IL-6) Interactions

Two related elements are present in the promoters of genes encoding acute phase proteins that mediate an increase in transcription stimulated by an IL-6 triggered signalling cascade (Hocke et al., 1992). One of these was found to be present in the exon 1B flanking sequence. Wild type and mutated reporter fusions for IL-6 were therefore tested for responsiveness in appropriate cell systems.

Constructs of the 1.5F B promoter normal and mutant (consensus change : ctggaa > gatatc[a] concerning the mutant: Hattori M et al (1990)*Proc. Natl. Acad. Sci. U S A.* Mar;87(6):2364-8.) were introduced into a promoter-less luciferase reporter vector and transfected into IN157 cells with a renilla firefly control. Cells were washed and charcoal stripped serum added 5 hours

post-transfection and left overnight. IL-6 amounts were added as illustrated with an appropriate amount of IL-6 soluble receptor. The cells were left for 24 hours and then assayed for activity using a luminometer.

A dosage dependent transcriptional response was noted in the normal, but not the mutated reporter construct (figure 10). This result indicates the existence of a cytokine mediated signalling pathway which causes up-regulation of the B utrophin promoter through the interaction of IL-6 and IL-6 receptor with the conserved IL-6 response element.

Discussion

We have demonstrated that there is a second promoter within intron 2 of the utrophin gene, driving expression of a unique first exon that splices into a common 13kb mRNA. These data are important, both in terms of understanding the molecular physiology of utrophin expression, and in view of their application to therapeutic intervention in DMD.

The functional consequences of genes having more than one promoter have been postulated (reviewed in (Ayoubi, *et al* (1996) *FASEB J.* **10**,453-460). A single gene may achieve a complex temporal and spatial expression pattern by interaction of different promoters with discrete subsets of transcription factors. Dystrophin is an example: three dissimilar promoters are active at different levels in specific cell types within the heart, skeletal muscle and the brain (Gorecki, *et al.* (1992) *Hum Mol Genet* **1**, 505-510., Barnea, *et al.* (1990) *Neuron* **5**, 881-888, Holder, *et al. Human Genetics* **97**, 232-239). Northern blot analysis, however, indicates that utrophin exon 1B is ubiquitously expressed, implying that promoters A and B are co-expressed in many tissues. It is conceivable that examination of transcript distribution in whole tissue samples has masked cell type-specific patterns of expression. Data

from isolated human cell lines *in vitro* support this notion; we observed large differences in promoter B activity between different cell lines, consistent with an *in vivo* expression profile involving specific cellular populations. Alternatively, the two promoters may be spatially regulated at a sub-cellular level. Within adult skeletal muscle fibres, promoter A is synaptically driven (Gramolini, *et al.* (1997) *J Biol Chem* **272**, 8117-20.), yet aggregates of utrophin mRNA are detectable at up to 25% extrasynaptic nuclei (Vater, *et al.* (1998) *Molecular and cellular Neuroscience* **10**, 229-242). Expression of promoter B in the extrasynaptic compartment might be invoked as one possible explanation.

A second proposed function of alternative promoters is the generation of transcripts with interchangeable 5' exons, giving rise to mRNAs with alternative 5'UTRs or proteins with novel N-terminal domains. Unlike exon 1B, utrophin exon 1A contains a long GC-rich 5'UTR. In some transcripts, GC-rich 5'UTRs are not translated efficiently (Kozak, M. (1991) *J Cell Biol* 115, 887-903.), and there are examples of genes in which alternative use of GC-rich and non-GC-rich 5'UTRs has been implicated in post-transcriptional regulation of protein synthesis (Nielson, et al. (1990) *J Biol Chem 265*, 13431-13434.). In addition, the predicted 31 amino acids encoded by exon 1B are different to the 26 amino acids of exon 2A; the functions of the resulting N-termini may be different.

The discovery of a second promoter provides a new target for the upregulation of utrophin to ameliorate the DMD phenotype. Promoter B is highly regulated, probably by different factors from promoter A, including IL-6. Elucidation of the mechanisms responsible for the large difference in promoter B activity between IN157 and HeLa cells might lead to identification of a factor that can be delivered to muscle to activate utrophin expression. Importantly, as the N-box motif is absent from promoter B, this is unlikely to carry any risk of NMJ

disruption potentially inherent in the pharmacological manipulation of synaptically regulated promoter A.

CLAIMS

1. An isolated nucleic acid comprising a promoter which comprises a sequence of nucleotides selected from (i) the human promoter sequence shown in Figure 1 and (ii) the mouse promoter sequence shown in Figure 2, free or substantially free of utrophin coding sequence.

2. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1440 in Figure 1.

3. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1183 of the mouse sequence shown in Figure 2.

4. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 1199 -1440 in the sequence shown in Figure 1.

5. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 959-1183 in the sequence shown in Figure 2.

6. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotide sequence ACAGGACATCCCAGTGTGCAGTTCG.

7. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 1, which sequence has at least 60% homology with the promoter sequence shown in figure 1 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that

sequence, said transcription being muscle-specific.

8. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which sequence has at least 60% homology with the promoter sequence shown in figure 2 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

9. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which hybridises to the promoter sequence shown in figure 2 under stringent hybridisation conditions and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

10. A nucleic acid construct comprising an isolated nucleic acid according to any of the preceding claims operably linked to a heterologous sequence.

11. A nucleic acid construct according to claim 10 wherein the heterologous sequence is a coding sequence.

12. A nucleic acid construct according to claim 11 wherein the heterologous sequence encodes a reporter molecule.

13. A host cell comprising a nucleic acid construct according to any of claims 10 to 12.

14. A method comprising culturing a host cell according to claim 13 under conditions for transcription of said heterologous sequence from the promoter.

15. A method according to claim 14 wherein the heterologous sequence is a coding sequence and the host cell is cultured under conditions for expression of the encoded peptide or polypeptide product.

16. A method according to claim 14 or claim 15 comprising detection of transcription of the heterologous sequence.

17. A method according to claim 14 or claim 15 comprising detection of expression of the encoded peptide or polypeptide product.

18. A method of screening for a substance able to modulate utrophin promoter activity, the method comprising contacting an expression system containing a nucleic acid construct according to any of claims 10 to 12 with a test or candidate substance and determining transcription of the heterologous sequence or expression of the encoded peptide or polypeptide product.

19. A method according to claim 18 wherein the expression system comprises a host cell containing said nucleic acid construct.

20. A method which comprises, following identification of a substance able to modulate utrophin promoter activity in accordance with a method according to claim 18 or claim 19, manufacture of the substance and/or use of the substance in manufacture or formulation of a composition.

21. The use of an isolated nucleic acid according to any of claims 1 to 6 for promoting transcription of an operably

linked sequence of nucleotides.

22. The use of claim 21 wherein the transcription is tissue-specific, with the tissue-specificity being muscle-specific.

23. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide including the amino acid sequence shown in Figure 1 or Figure 2.

24. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide including the amino acid sequence shown in Figure 1, which amino acid sequence has at least 60% homology with the polypeptide sequence in Figure 1 or Figure 2.

25. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide shown in Figure 1 or Figure 2, which nucleotide sequence hybridises with the nucleotide sequence encoding the polypeptide in Figure 1 or Figure 2 under stringent hybridisation conditions.

26. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence shown in Figure 9.

27. An isolated nucleic acid molecule comprising the nucleotide sequence shown in figure 9.

28. Nucleic acid of any one of claims 23 to 27 comprised in a vector.

29. Nucleic acid according to claim 28 wherein said vector is an expression vector.

30. A host cell containing heterologous nucleic acid according to any one of claims 23 to 29.

31. A cell according to claim 30 which is a muscle cell.

32. A cell according to claim 30 wherein said polypeptide is expressed.

33. A cell according to any of claims 30 to 32 which is in a mammal.

34. A non-human mammal having a cell according to any of claims 30 to 32.

35. A non-human mammal containing nucleic acid according to any of claims 23 to 29.

36. A method including introduction of nucleic acid according to any of claims 23 to 29 into a cell.

37. A method according to claim 36 wherein said introduction takes place *in vitro*.

38. A method which includes causing or allowing expression of the coding nucleotide sequence of heterologous nucleic acid according to any of claims 23 to 29 in a cell.

39. A method according to claim 38 wherein the cell is part of a mammal.

40. A method according to claim 38 wherein the expression product is purified and/or isolated following expression.

41. A method according to claim 40 wherein the expression product is formulated into a composition which includes at least one additional component, following purification and/or

isolation of the expression product.

42. An isolated polypeptide as encoded by nucleic acid according to any of claims 23 to 29.

43. An isolated utrophin exon IB polypeptide selected from:
(i) human utrophin exon IB polypeptide of which the amino acid sequence is shown in Figure 1;
(ii) mouse utrophin exon IB of which the amino acid sequence is shown in Figure 1.

44. An isolated polypeptide including the human polypeptide according to claim 43.

45. An isolated polypeptide including the mouse polypeptide according to claim 44.

46. An isolated polypeptide which has 60 % homology with the polypeptide according to claim 44 or 45.

47. An isolated fragment of a polypeptide according to claim 43, which fragments is 5 to 25 amino acids in length.

48. An isolated fragment of a polypeptide according to claim 43, which fragment is 10 to 20 amino acids in length.

49. An antibody specific for a polypeptide according to any one of claims 42 to 48.

50. A composition including a polypeptide according to any one of claims 42 to 46, a fragment according to claim 47 or claim 48, or an antibody according to claim 49, and a pharmaceutically acceptable excipient.

51. Use of nucleic acid according to any of claims 23 to 29 in the manufacture of a medicament for treating a dystrophin

phenotype in a mammal.

52. Use of a polypeptide according to any of claims 42 to 48 or an antibody according to claim 49 in the manufacture of a medicament for treating a dystrophin phenotype in a mammal.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number
WO 01/25461 A1

(51) International Patent Classification[7]: C12N 15/85, 15/12, A01K 67/027, C07K 14/47, 16/18, A61K 38/17

(21) International Application Number: PCT/GB00/03800

(22) International Filing Date: 4 October 2000 (04.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9923423.9 4 October 1999 (04.10.1999) GB

(71) Applicant *(for all designated States except US)*: **ISIS INNOVATION LIMITED** [GB/GB]; Ewert House, Ewert Place, Summertown, Oxford OX2 7DD (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BURTON, Edward** [GB/US]; University of Pittsburgh, School of Medicine, Department of Molecular Genetics and Biochemistry, E1215 Biomedical Science Tower, Pittsburg, PA 15261 (US). **TINSLEY, Jonathan** [GB/GB]; 5 Spruce Gardens, Oxford OX4 7GH (GB). **DAVIES, Kay** [GB/GB]; 55 Five Mile Drive, Oxford OX2 8HR (GB).

(74) Agents: **WALTON, Sean, M.** et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

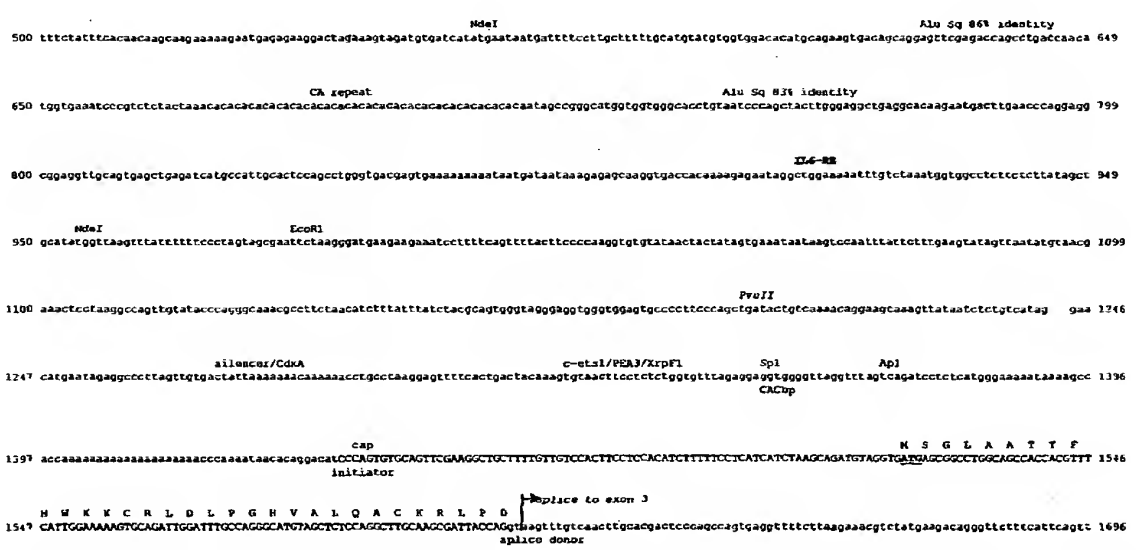
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

[Continued on next page]

(54) Title: UTROPHIN GENE PROMOTER



(57) Abstract: Second promoter for mouse and human utrophin genes. The promoters or fragments and derivatives may be used to control transcription of heterologous sequences, including coding sequences of reporter genes. Expression systems such as host cells containing nucleic acid constructs which comprise a promoter as provided operably linked to a heterologous sequence may be used to screen substances for ability to modulate activity of the utrophin promoter. Substances with such ability may be manufactured and/or used in the preparation of compositions such as medicaments. Up-regulation of utrophin expression may compensate for dystrophin loss in muscular dystrophy patients.

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```
                                                  NdeI                                                       Alu Sq 86% identity
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                         CA repeat                                                    Alu Sq 83% identity
650  tggtgaaatcccgtctctactaaacacacacacacacacacacacacacacacacacacacacaatagccgggcatggtggtgggcacctgtaatcccagctacttgggaggctgaggcacaagaatgacttgaacccaggagg 799

                                                                                                                   IL6-RE
800  cggaggttgcagtgagctgagatcatgccattgcactccagcctgggtgacgagtgaaaaaaaaataatgataataaagagagcaaggtgaccacaaaagagaataggctggaaaaatttgtctaaatggtggcctcttctcttatagct 949

     NdeI                 EcoR1
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                                                                                                       PvuII
1100 aaactcctaaggccagttgtatacccagggcaaacgccttctaacatctttatttatctacgcagtgggtagggaggtgggtggagtgccccttcccagctgatactgtcaaaacaggaagcaaagttataatctctgtcatag   gaa 1246

                     silencer/CdxA                                          c-ets1/PEA3/XrpF1        Sp1            Ap1
1247 catgaatagaggcccttagttgtgactattaaaaaaacaaaaaacctgcctaaggagttttcactgactacaaagtgtaacttcctctctggtgtttagaggaggtggggttaggtttagtcagatcctctcatgggaaaaataaaagcc 1396
                                                                                                     CACbp

                                   cap                                                                                           M  S  G  L  A  A  T  T  F
1397 accaaaaaaaaaaaaaaaaaaaacccaaaataacacaggacatCCCAGTGTGCAGTTCGAAGGCTGCTTTTGTTGTCCACTTCCTCCACATCTTTTTCCTCATCATCTAAGCAGATGTAGGTGATGAGCGGCCTGGCAGCCACCACGTTT 1546
                                 initiator

                                                                   |→splice to exon 3
     H  W  K  K  C  R  L  D  L  P  G  H  V  A  L  Q  A  C  K  R  L  F  D
1547 CATTGGAAAAAGTGCAGATTGGATTTGCCAGGGCATGTAGCTCTCCAGGCTTGCAAGCGATTACCAGgtaagtttgtcaacttgcacgactcccagccagtgaggttttcttaagaaacgtctatgaagacagggttctttcattcagtt 1696
                                                                   splice donor
```

Figure 1

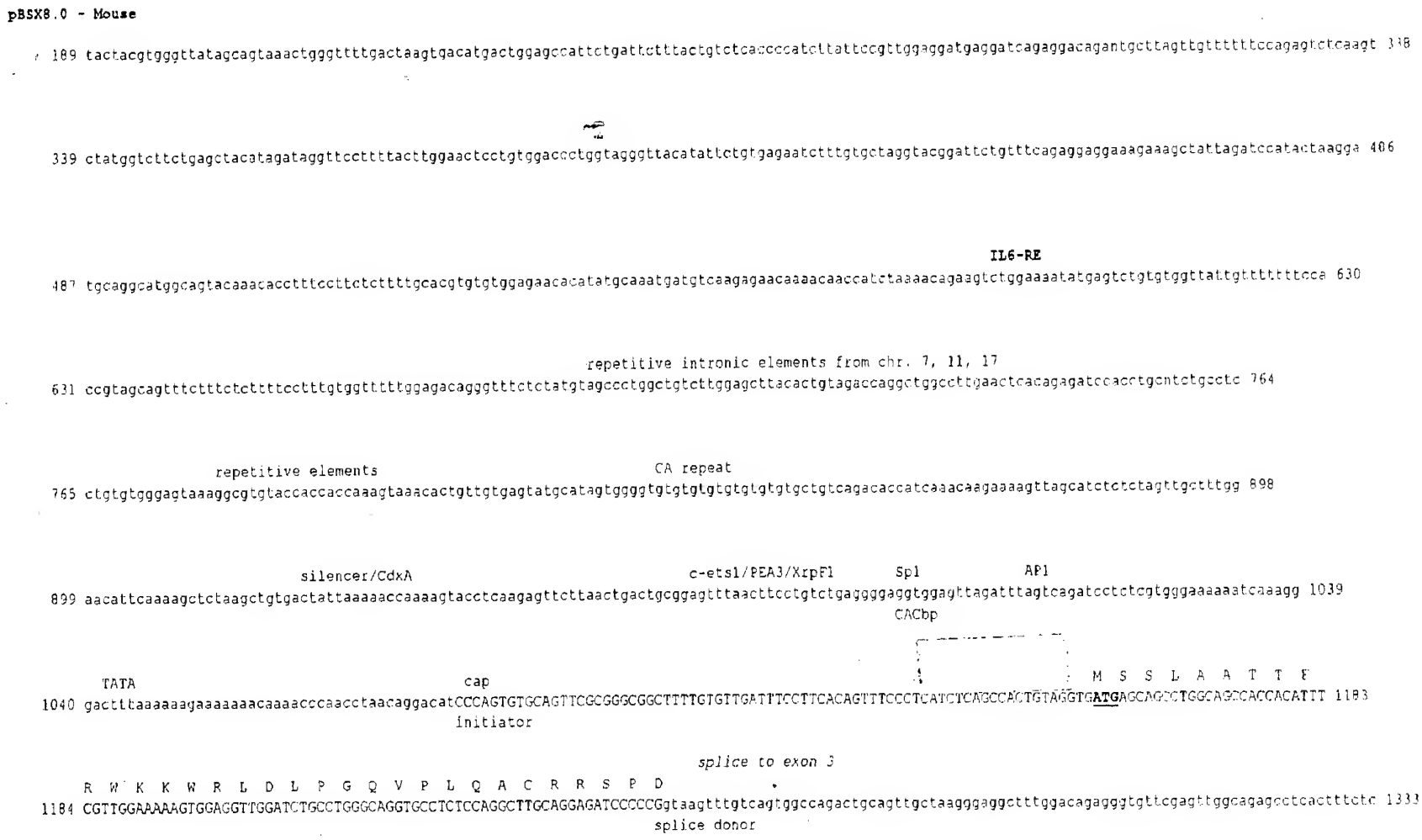
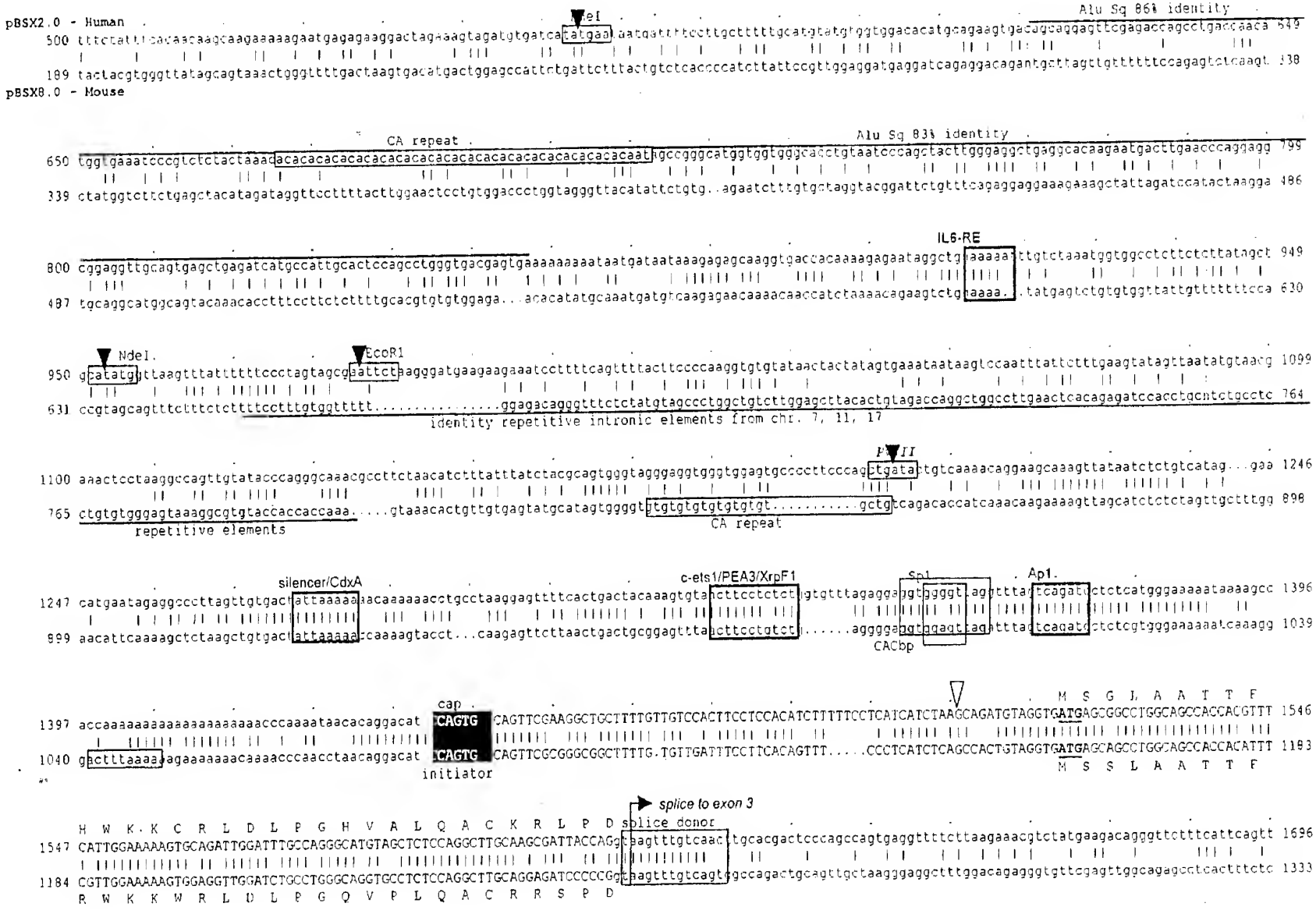


Figure 2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pBSX8.0 - Mouse
Alu Sq 86% identity
Alu Sq 83% identity
CA repeat
IL6-RE
NdeI
EcoR1
identity repetitive intronic elements from chr. 7, 11, 17
repetitive elements
CA repeat
silencer/CdxA
c-ets1/PEA3/XrpF1
Sp1
Ap1
CACbp
cap
initiator
splice to exon 3
splice donor

Figure 3

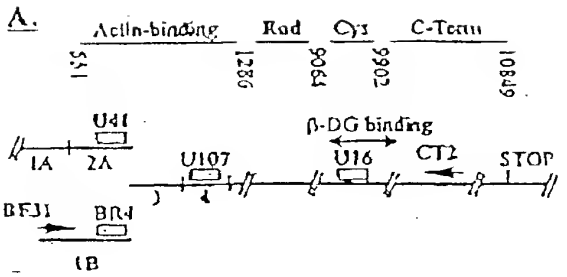
A.
Actin-binding
Rod
Cys
C-Term
151
1289
9864
9902
10849
U41
1A
2A
U107
β-DG binding
U16
CT2
STOP
BF31
BR4
1B

Figure 4

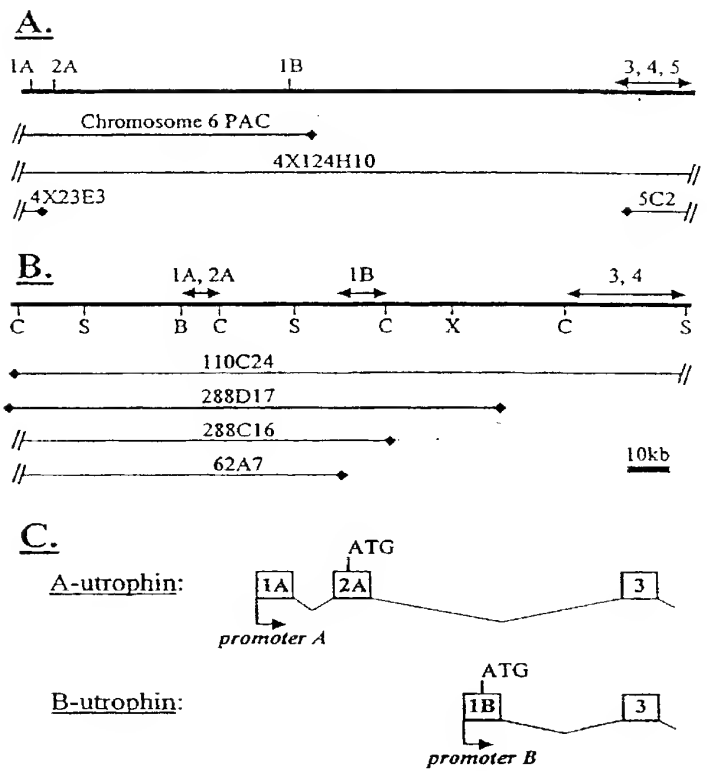
A.
1A 2A
1B
3, 4, 5
Chromosome 6 PAC
4X124H10
4X23E3
5C2
B.
1A, 2A
1B
3, 4
C S B C S C X C S
110C24
288D17
288C16
62A7
10kb
C.
A-utrophin:
ATG
1A
2A
3
promoter A
B-utrophin:
ATG
1B
3
promoter B

Figure 5

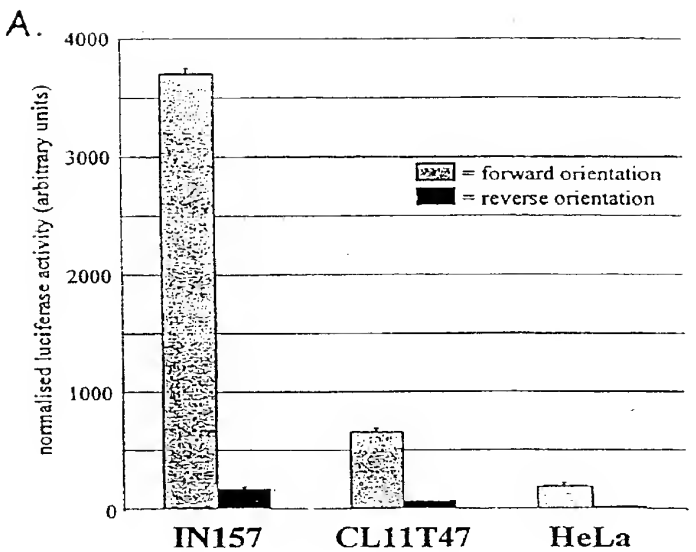
A.
4000
3000
2000
1000
0
normalised luciferase activity (arbitrary units)
= forward orientation
= reverse orientation
IN157
CL11T47
HeLa

Figure 6

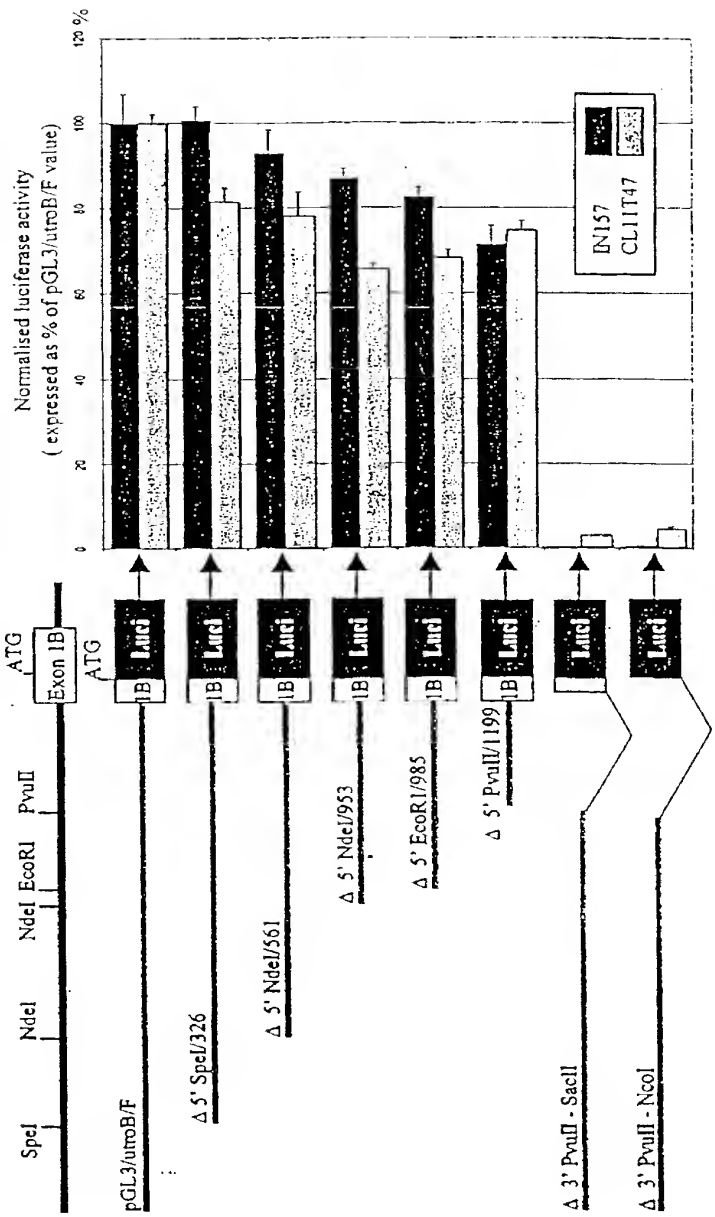
Normalised luciferase activity
(expressed as % of pGL3/utroB/F value)
120 %
100
80
60
40
20
0
IN157
CL11T47
ATG
Exon 1B
ATG
LucI
1B
SpeI
NdeI
NdeI
EcoRI
PvuII
pGL3/utroB/F
Δ 5' SpeI/326
Δ 5' NdeI/561
Δ 5' NdeI/953
Δ 5' EcoR1/985
Δ 5' PvuII/1199
Δ 3' PvuII - SacII
Δ 3' PvuII - NcoI

Figure 7

Human B-utrophin up to nucleotide 1500, deduced translation

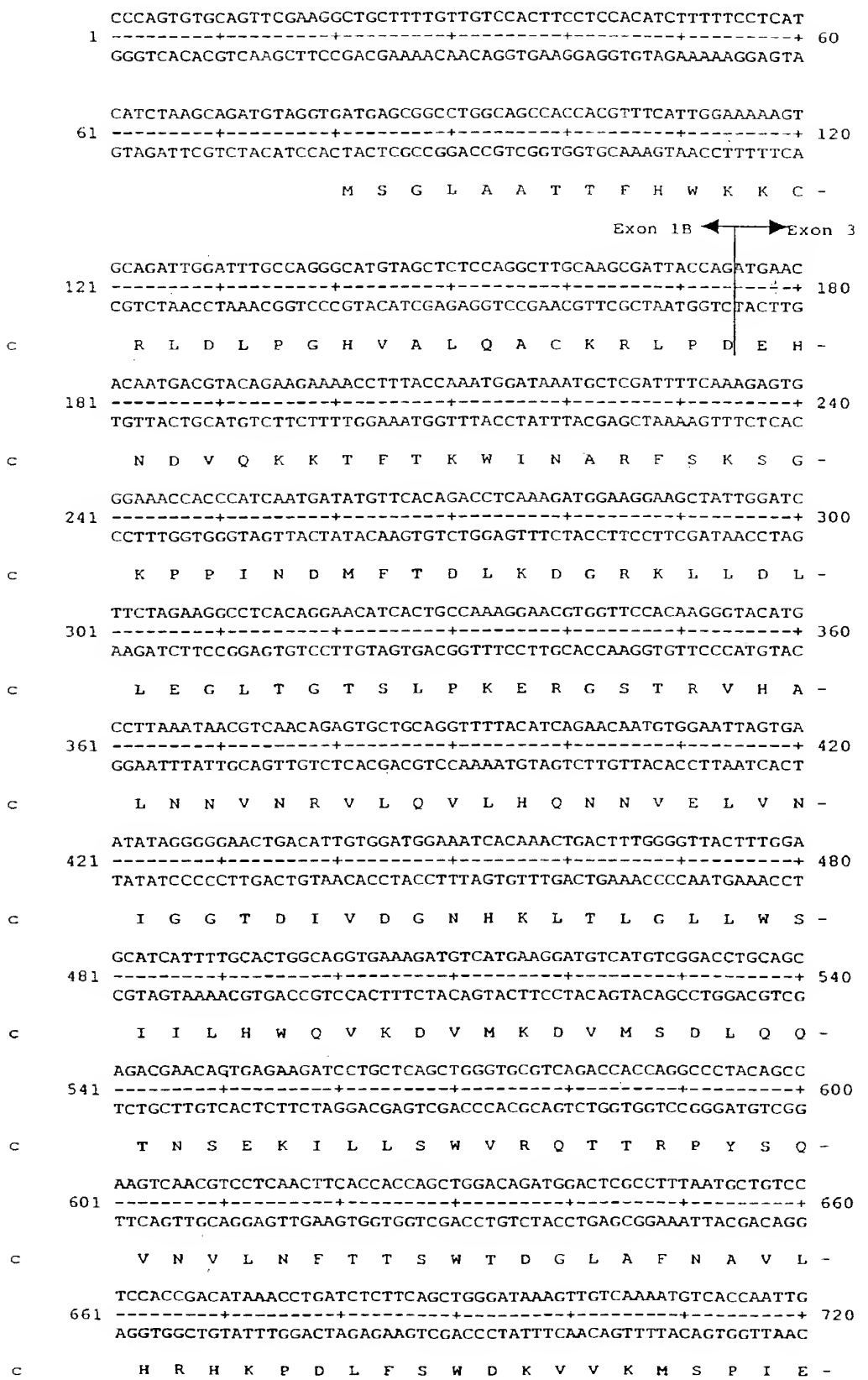

Figure 8

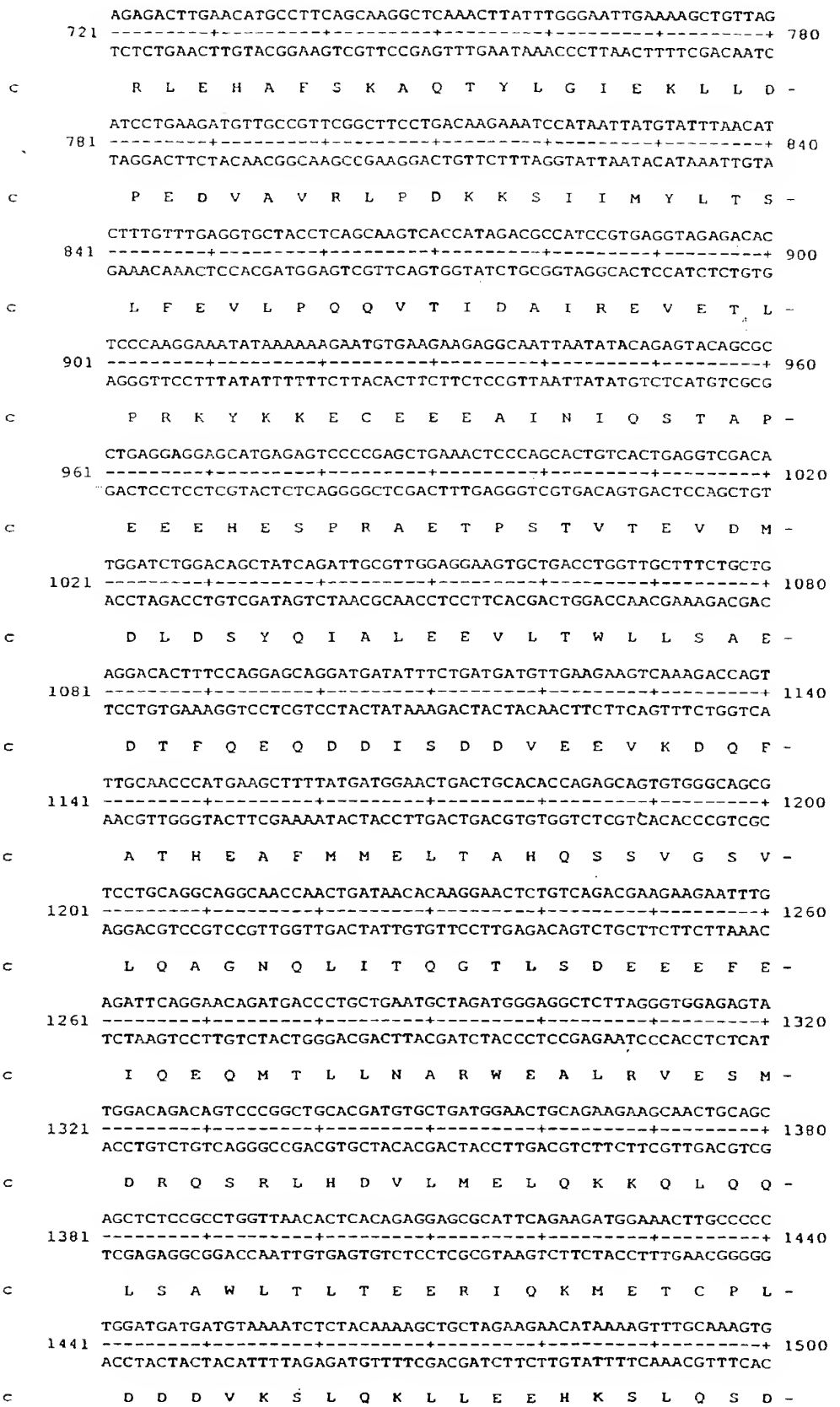

Figure 8 cont ...

Sequence Range: 1 to 6059

```
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           M  S  G  L  A  A  T  T  F  H  W  K  K  C  R  L  D  L  P  G  H  V  A>

        90        100        110        120        130        140        150        160
TCCAGGCTTG CAAGCGATTA CCAGATGAAC ACAATGATGT ACAGAAGAAA ACCTTTACCA AATGGATAAA CGCTCGATTT
L  Q  A  C  K  R  L  P  D  E  H  N  D  V  Q  K  K  T  F  T  K  W  I  N  A  R  F>

       170        180        190        200        210        220        230        240
TCCAAGAGTG GGAAACCACC CATCAGTGAT ATGTTCTCAG ACCTCAAAGA TGGGAGAAAG CTCTTGGATC TTCTCGAAGG
 S  K  S  G  K  P  P  I  S  D  M  F  S  D  L  K  D  G  R  K  L  L  D  L  L  E  G>

       250        260        270        280        290        300        310        320
CCTCACAGGA ACATCATTGC CAAAGGAACG TGGTTCCACA AGGGTGCATG CCTTAAACAA TGTCAACCGA GTGCTACAGG
  L  T  G  T  S  L  P  K  E  R  G  S  T  R  V  H  A  L  N  N  V  N  R  V  L  Q>

       330        340        350        360        370        380        390        400
TTTTACATCA GAACAATGTG GACTTGGTGA ATATTGGAGG CACGGACATT GTGGCTGGAA ATCCCAAGCT GACTTTAGGG
V  L  H  Q  N  N  V  D  L  V  N  I  G  G  T  D  I  V  A  G  N  P  K  L  T  L  G>

       410        420        430        440        450        460        470        480
TTACTCTGGA GCATCATTCT GCACTGGCAG GTGAAGGATG TCATGAAAGA TATCATGTCA GACCTGCAGC AGACAAACAG
 L  L  W  S  I  I  L  H  W  Q  V  K  D  V  M  K  D  I  M  S  D  L  Q  Q  T  N  S>

       490        500        510        520        530        540        550        560
CGAGAAGATC CTGCTGAGCT GGGTGCGGCA GACCACCAGG CCCTACAGTC AAGTCAACGT CCTCAACTTC ACCACCAGCT
  E  K  I  L  L  S  W  V  R  Q  T  T  R  P  Y  S  Q  V  N  V  L  N  F  T  T  S>

       570        580        590        600        610        620        630        640
GGACCGATGG ACTCGCGTTC AACGCCGTGC TCCACCGGCA CAAACCAGAT CTCTTCGACT GGGACGAGAT GGTCAAAATG
W  T  D  G  L  A  F  N  A  V  L  H  R  H  K  P  D  L  F  D  W  D  E  M  V  K  M>

       650        660        670        680        690        700        710        720
TCCCCAATTG AGAGACTTGA CCATGCTTTT GACAAGGCCC ACACTTCTTT GGGAATTGAA AAGCTCCTAA GTCCTGAAAC
 S  P  I  E  R  L  D  H  A  F  D  K  A  H  T  S  L  G  I  E  K  L  L  S  P  E  T>

       730        740        750        760        770        780        790        800
TGTTGCTGTG CATCTCCCTG ACAAGAAATC CATAATTATG TATTTAACGT CTCTGTTTGA GGTGCTTCCT CAGCAAGTCA
  V  A  V  H  L  P  D  K  K  S  I  I  M  Y  L  T  S  L  F  E  V  L  P  Q  Q  V>

       810        820        830        840        850        860        870        880
CGATAGATGC CATCCGAGAG GTGGAGACTC TCCCAAGGAA GTATAAGAAA GAATGTGAAG AGGAAGAAAT TCATATCCAG
T  I  D  A  I  R  E  V  E  T  L  P  R  K  Y  K  K  E  C  E  E  E  E  I  H  I  Q>

       890        900        910        920        930        940        950        960
AGTGCAGTGC TGGCAGAGGA AGGCCAGAGT CCCCGAGCTG AGACCCCTAG CACCGTCACT GAAGTGGACA TGGATTTGGA
 S  A  V  L  A  E  E  G  Q  S  P  R  A  E  T  P  S  T  V  T  E  V  D  M  D  L  D>

       970        980        990       1000       1010       1020       1030       1040
CAGCTACCAG ATAGCGCTAG AGGAAGTGCT GACGTGGCTG CTGTCCGCGG AGGACACGTT CCAGGAGCAA CATGACATTT
 S  Y  Q  I  A  L  E  E  V  L  T  W  L  L  S  A  E  D  T  F  Q  E  Q  H  D  I>

      1050       1060       1070       1080       1090       1100       1110       1120
CTGATGATGT CGAAGAAGTC AAAGAGCAGT TTGCTACCCA TGAAACTTTT ATGATGGAGC TGACAGCACA CCAGAGCAGC
S  D  D  V  E  E  V  K  E  Q  F  A  T  H  E  T  F  M  M  E  L  T  A  H  Q  S  S>

      1130       1140       1150       1160       1170       1180       1190       1200
GTGGGGAGCG TCCTGCAGGC TGGCAACCAG CTGATGACAC AAGGGACTCT GTCCAGAGAG GAGGAGTTTG AGATCCAGGA
 V  G  S  V  L  Q  A  G  N  Q  L  M  T  Q  G  T  L  S  R  E  E  E  F  E  I  Q  E>

      1210       1220       1230       1240       1250       1260       1270       1280
ACAGATGACC TTGCTGAATG CAAGGTGGGA GGCGCTCCGG GTGGAGAGCA TGGAGAGGCA GTCCCGGCTG CACGACGCTC
  Q  M  T  L  L  N  A  R  W  E  A  L  R  V  E  S  M  E  R  Q  S  R  L  H  D  A>

      1290       1300       1310       1320       1330       1340       1350       1360
TGATGGAGCT GCAGAAGAAA CAGCTGCAGC AGCTCTCAAG CTGGCTGGCC CTCACAGAAG AGCGCATTCA GAAGATGGAG
```

Figure 9

```
L  M  E  L  Q  K  K  Q  L  Q  Q  L  S  S  W  L  A  L  T  E  E  R  I  Q  K  M  E>

      1370       1380       1390       1400       1410       1420       1430       1440
AGCCTCCCGC TGGGTGATGA CCTGCCCTCC CTGCAGAAGC TGCTTCAAGA ACATAAAAGT TTGCAAAATG ACCTTGAAGC
 S  L  P  L  G  D  D  L  P  S  L  Q  K  L  L  Q  E  H  K  S  L  Q  N  D  L  E  A>

      1450       1460       1470       1480       1490       1500       1510       1520
TGAACAGGTG AAGGTAAATT CCTTAACTCA CATGGTGGTG ATTGTGGATG AAAACAGTGG GGAGAGTGCC ACAGCTCTTC
   E  Q  V  K  V  N  S  L  T  H  M  V  V  I  V  D  E  N  S  G  E  S  A  T  A  L>

      1530       1540       1550       1560       1570       1580       1590       1600
TGGAAGATCA GTTACAGAAA CTGGGTGAGC GCTGGACAGC TGTATGCCGC TGGACTGAAG AACGTTGGAA CAGGTTGCAA
L  E  D  Q  L  Q  K  L  G  E  R  W  T  A  V  C  R  W  T  E  E  R  W  N  R  L  Q>

      1610       1620       1630       1640       1650       1660       1670       1680
GAAATCAGTA TTCTGTGGCA GGAATTATTG GAAGAGCAGT GTCTGTTGGA GGCTTGGCTC ACCGAAAAGG AAGAGGCTTT
 E  I  S  I  L  W  Q  E  L  L  E  E  Q  C  L  L  E  A  W  L  T  E  K  E  E  A  L>

      1690       1700       1710       1720       1730       1740       1750       1760
GGATAAAGTT CAAACCAGCA ACTTTAAAGA CCAGAAGGAA CTAAGTGTCA GTGTCCGGCG TCTGGCTATA TTGAAGGAAG
  D  K  V  Q  T  S  N  F  K  D  Q  K  E  L  S  V  S  V  R  R  L  A  I  L  K  E>

      1770       1780       1790       1800       1810       1820       1830       1840
ACATGGAAAT GAAGAGGCAG ACTCTGGATC AACTGAGTGA GATTGGCCAG GATGTGGGCC AATTACTCAG TAATCCCAAG
D  M  E  M  K  R  Q  T  L  D  Q  L  S  E  I  G  Q  D  V  G  Q  L  L  S  N  P  K>

      1850       1860       1870       1880       1890       1900       1910       1920
GCATCTAAGA AGATGAACAG TGACTCTGAG GAGCTAACAC AGAGATGGGA TTCTCTGGTT CAGAGACTCG AAGACTCTTC
 A  S  K  K  M  N  S  D  S  E  E  L  T  Q  R  W  D  S  L  V  Q  R  L  E  D  S  S>

      1930       1940       1950       1960       1970       1980       1990       2000
TAACCAGGTG ACTCAGGCGG TAGCGAAGCT CGGCATGTCC CAGATTCCAC AGAAGGACCT ATTGGAGACC GTTCATGTGA
  N  Q  V  T  Q  A  V  A  K  L  G  M  S  Q  I  P  Q  K  D  L  L  E  T  V  H  V>

      2010       2020       2030       2040       2050       2060       2070       2080
GAGAACAAGG GATGGTGAAG AAGCCCAAGC AGGAACTGCC TCCTCCGTTA ACAAAGGCTG AGCATGCTAT GCAAAAGAGA
R  E  Q  G  M  V  K  K  P  K  Q  E  L  P  P  P  L  T  K  A  E  H  A  M  Q  K  R>

      2090       2100       2110       2120       2130       2140       2150       2160
TCAACCACCG AATTGGGAGA AAACCTGCAA GAATTAAGAG ACTTAACTCA AGAAATGGAA GTACATGCTG AAAAACTCAA
 S  T  T  E  L  G  E  N  L  Q  E  L  R  D  L  T  Q  E  M  E  V  H  A  E  K  L  K>

      2170       2180       2190       2200       2210       2220       2230       2240
ATGGCTGAAT AGAACTGAAT TGGAGATGCT TTCAGATAAA AGTCTGAGTT TACCTGAAAG GGATAAAATT TCAGAAAGCT
  W  L  N  R  T  E  L  E  M  L  S  D  K  S  L  S  L  P  E  R  D  K  I  S  E  S>

      2250       2260       2270       2280       2290       2300       2310       2320
TAAGGACTGT AAATATGACA TGGAATAAGA TTTGCAGAGA GGTGCCTACC ACCCTGAAGG AATGCATCCA GGAGCCCAGT
L  R  T  V  N  M  T  W  N  K  I  C  R  E  V  P  T  T  L  K  E  C  I  Q  E  P  S>

      2330       2340       2350       2360       2370       2380       2390       2400
TCTGTTTCAC AGACAAGGAT TGCTGCTCAT CCTAATGTCC AAAAGGTGGT GCTAGTATCA TCTGCGTCAG ATATTCCTGT
 S  V  S  Q  T  R  I  A  A  H  P  N  V  Q  K  V  V  L  V  S  S  A  S  D  I  P  V>

      2410       2420       2430       2440       2450       2460       2470       2480
TCAGTCTCAT CGTACTTCGG AAATTTCAAT TCCTGCTGAT CTTGATAAAA CTATAACAGA ACTAGCCGAC TGGCTGGTAT
  Q  S  H  R  T  S  E  I  S  I  P  A  D  L  D  K  T  I  T  E  L  A  D  W  L  V>

      2490       2500       2510       2520       2530       2540       2550       2560
TAATCGACCA GATGCTGAAG TCCAACATTG TCACTGTTGG GGATGTAGAA GAGATCAATA AGACCGTTTC CCGAATGAAA
L  I  D  Q  M  L  K  S  N  I  V  T  V  G  D  V  E  E  I  N  K  T  V  S  R  M  K>

      2570       2580       2590       2600       2610       2620       2630       2640
ATTACAAAGG CTGACTTAGA ACAGCGCCAT CCTCAGCTGG ATTATGTTTT TACATTGGCA CAGAATTTGA AAAATAAAGC
 I  T  K  A  D  L  E  Q  R  H  P  Q  L  D  Y  V  F  T  L  A  Q  N  L  K  N  K  A>

      2650       2660       2670       2680       2690       2700       2710       2720
TTCCAGTTCA GATATGAGAA CAGCAATTAC AGAAAAAATTG GAAAGGGTCA AGAACCAGTG GGATGGCACC CAGCATGGCG
```

Figure 9 cont ...

```
  S  S  S  D  M  R  T  A  I  T  E  K  L  E  R  V  K  N  Q  W  D  G  T  Q  H  G>

      2730       2740       2750       2760       2770       2780       2790       2800
TTGAGCTAAG ACAGCAGCAG CTTGAGGACA TGATTATTGA CAGTCTTCAG TGGGATGACC ATAGGGAGGA GACTGAAGAA
 V  E  L  R  Q  Q  Q  L  E  D  M  I  I  D  S  L  Q  W  D  D  H  R  E  E  T  E  E>

      2810       2820       2830       2840       2850       2860       2870       2880
CTGATGAGAA AATATGAGGC TCGACTCTAT ATTCTTCAGC AAGCCCGACG GGATCCACTC ACCAAACAAA TTTCTGATAA
 L  M  R  K  Y  E  A  R  L  Y  I  L  Q  Q  A  R  R  D  P  L  T  K  Q  I  S  D  N>

      2890       2900       2910       2920       2930       2940       2950       2960
CCAAATACTG CTTCAAGAAC TGGGTCCTGG AGATGGTATC GTCATGGCGT TCGATAACGT CCTGCAGAAA CTCCTGGAGG
 Q  I  L  L  Q  E  L  G  P  G  D  G  I  V  M  A  F  D  N  V  L  Q  K  L  L  E>

      2970       2980       2990       3000       3010       3020       3030       3040
AATATGGGAG TGATGACACA AGGAATGTGA AAGAAACCAC AGAGTACTTA AAAACATCAT GGATCAATCT CAAACAAAGT
 E  Y  G  S  D  D  T  R  N  V  K  E  T  T  E  Y  L  K  T  S  W  I  N  L  K  Q  S>

      3050       3060       3070       3080       3090       3100       3110       3120
ATTGCTGACA GACAGAACGC CTTGGAGGCT GAGTGGAGGA CGGTGCAGGC CTCTCGCAGA GATCTGGAAA ACTTCCTGAA
 I  A  D  R  Q  N  A  L  E  A  E  W  R  T  V  Q  A  S  R  R  D  L  E  N  F  L  K>

      3130       3140       3150       3160       3170       3180       3190       3200
GTGGATCCAA GAAGCAGAGA CCACAGTGAA TGTGCTTGTG GATGCCTCTC ATCGGGAGAA TGCTCTTCAG GATAGTATCT
 W  I  Q  E  A  E  T  T  V  N  V  L  V  D  A  S  H  R  E  N  A  L  Q  D  S  I>

      3210       3220       3230       3240       3250       3260       3270       3280
TGGCCAGGGA ACTCAAACAG CAGATGCAGG ACATCCAGGC AGAAATTGAT GCCCACAATG ACATATTTAA AAGCATTGAC
 L  A  R  E  L  K  Q  Q  M  Q  D  I  Q  A  E  I  D  A  H  N  D  I  F  K  S  I  D>

      3290       3300       3310       3320       3330       3340       3350       3360
GGAAACAGGC AGAAGATGGT AAAAGCTTTG GGAAATTCTG AAGAGGCTAC TATGCTTCAA CATCGACTGG ATGATATGAA
 G  N  R  Q  K  M  V  K  A  L  G  N  S  E  E  A  T  M  L  Q  H  R  L  D  D  M  N>

      3370       3380       3390       3400       3410       3420       3430       3440
CCAAAGATGG AATGACTTAA AAGCAAAATC TGCTAGCATC AGGGCCCATT TGGAGGCCAG CGCTGAGAAG TGGAACAGGT
 Q  R  W  N  D  L  K  A  K  S  A  S  I  R  A  H  L  E  A  S  A  E  K  W  N  R>

      3450       3460       3470       3480       3490       3500       3510       3520
TGCTGATGTC CTTAGAAGAA CTGATCAAAT GGCTGAATAT GAAAGATGAA GAGCTTAAGA AACAAATGCC TATTGGAGGA
 L  L  M  S  L  E  E  L  I  K  W  L  N  M  K  D  E  E  L  K  K  Q  M  P  I  G  G>

      3530       3540       3550       3560       3570       3580       3590       3600
GATGTTCCAG CCTTACAGCT CCAGTATGAC CATTGTAAGG CCCTGAGACG GGAGTTAAAG GAGAAAGAAT ATTCTGTCCT
 D  V  P  A  L  Q  L  Q  Y  D  H  C  K  A  L  R  R  E  L  K  E  K  E  Y  S  V  L>

      3610       3620       3630       3640       3650       3660       3670       3680
GAATGCTGTC GACCAGGCCC GAGTTTTCTT GGCTGATCAG CCAATTGAGG CCCCTGAAGA GCCAAGAAGA AACCTACAAT
 N  A  V  D  Q  A  R  V  F  L  A  D  Q  P  I  E  A  P  E  E  P  R  R  N  L  Q>

      3690       3700       3710       3720       3730       3740       3750       3760
CAAAAACAGA ATTAACTCCT GAGGAGAGAG CCCAAAAGAT TGCCAAAGCC ATGCGCAAAC AGTCTTCTGA AGTCAAAGAA
 S  K  T  E  L  T  P  E  E  R  A  Q  K  I  A  K  A  M  R  K  Q  S  S  E  V  K  E>

      3770       3780       3790       3800       3810       3820       3830       3840
AAATGGGAAA GTCTAAATGC TGTAACTAGC AATTGGCAAA AGCAAGTGGA CAAGGCATTG GAGAAACTCA GAGACCTGCA
 K  W  E  S  L  N  A  V  T  S  N  W  Q  K  Q  V  D  K  A  L  E  K  L  R  D  L  Q>

      3850       3860       3870       3880       3890       3900       3910       3920
GGGAGCTATG GATGACCTGG ACGCTGACAT GAAGGAGGCA GAGTCCGTGC GGAATGGCTG GAAGCCCGTG GGAGACTTAC
 G  A  M  D  D  L  D  A  D  M  K  E  A  E  S  V  R  N  G  W  K  P  V  G  D  L>

      3930       3940       3950       3960       3970       3980       3990       4000
TCATTGACTC GCTGCAGGAT CACATTGAAA AAATCATGGC ATTTAGAGAA GAAATTGCAC CAATCAACTT TAAAGTTAAA
 L  I  D  S  L  Q  D  H  I  E  K  I  M  A  F  R  E  E  I  A  P  I  N  F  K  V  K>

      4010       4020       4030       4040       4050       4060       4070       4080
ACGGTGAATG ATTTATCCAG TCAGCTGTCT CCACTTGACC TGCATCCCTC TCTAAAGATG TCTCGCCAGC TAGATGACCT
```

Figure 9 cont ...

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 T  V  N  D  L  S  S  Q  L  S  P  L  D  L  H  P  S  L  K  M  S  R  Q  L  D  D  L>

      4090       4100       4110       4120       4130       4140       4150       4160
TAATATGCGA TGGAAACTTT TACAGGTTTC TGTGGATGAT CGCCTTAAAC AGCTTCAGGA AGCCCACAGA GATTTTGGAC
  N  M  R  W  K  L  L  Q  V  S  V  D  D  R  L  K  Q  L  Q  E  A  H  R  D  F  G>

      4170       4180       4190       4200       4210       4220       4230       4240
CATCCTCTCA GCATTTTCTC TCTACGTCAG TCCAGCTGCC GTGGCAAAGA TCCATTTCAC ATAATAAAGT GCCCTATTAC
P  S  S  Q  H  F  L  S  T  S  V  Q  L  P  W  Q  R  S  I  S  H  N  K  V  P  Y  Y>

      4250       4260       4270       4280       4290       4300       4310       4320
ATCAACCATC AAACACAGAC CACCTGTTGG GACCATCCTA AAATGACCGA ACTCTTTCAA TCCCTTGCTG ACCTGAATAA
  I  N  H  Q  T  Q  T  T  C  W  D  H  P  K  M  T  E  L  F  Q  S  L  A  D  L  N  N>

      4330       4340       4350       4360       4370       4380       4390       4400
TGTACGTTTT TCTGCCTACC GTACAGCAAT CAAAATCCGA AGACTACAAA AAGCACTATG TTTGGATCTC TTAGAGTTGA
  V  R  F  S  A  Y  R  T  A  I  K  I  R  R  L  Q  K  A  L  C  L  D  L  L  E  L>

      4410       4420       4430       4440       4450       4460       4470       4480
GTACAACAAA TGAAATTTTC AAACAGCACA AGTTGAACCA AAATGACCAG CTCCTCAGTG TTCCAGATGT CATCAACTGT
S  T  T  N  E  I  F  K  Q  H  K  L  N  Q  N  D  Q  L  L  S  V  P  D  V  I  N  C>

      4490       4500       4510       4520       4530       4540       4550       4560
CTGACAACAA CTTATGATGG ACTTGAGCAA ATGCATAAGG ACCTGGTCAA CGTTCCACTC TGTGTTGATA TGTGTCTCAA
  L  T  T  T  Y  D  G  L  E  Q  M  H  K  D  L  V  N  V  P  L  C  V  D  M  C  L  N>

      4570       4580       4590       4600       4610       4620       4630       4640
TTGGTTGCTC AATGTCTATG ACACGGGTCG AACTGGAAAA ATTAGAGTGC AGAGTCTGAA GATTGGATTA ATGTCTCTCT
  W  L  L  N  V  Y  D  T  G  R  T  G  K  I  R  V  Q  S  L  K  I  G  L  M  S  L>

      4650       4660       4670       4680       4690       4700       4710       4720
CCAAAGGTCT CTTGGAAGAA AAATACAGAT ATCTCTTTAA GGAAGTTGCG GGGCCGACAG AAATGTGTGA CCAGAGGCAG
S  K  G  L  L  E  E  K  Y  R  Y  L  F  K  E  V  A  G  P  T  E  M  C  D  Q  R  Q>

      4730       4740       4750       4760       4770       4780       4790       4800
CTGGGCCTGT TACTTCATGA TGCCATCCAG ATCCCCCGGC AGCTAGGTGA AGTAGCAGCT TTTGGAGGCA GTAATATTGA
  L  G  L  L  L  H  D  A  I  Q  I  P  R  Q  L  G  E  V  A  A  F  G  G  S  N  I  E>

      4810       4820       4830       4840       4850       4860       4870       4880
GCCTAGTGTT CGCAGCTGCT TCCAACAGAA TAACAATAAA CCAGAAATAA GTGTGAAAGA GTTTATAGAT TGGATGCATT
  P  S  V  R  S  C  F  Q  Q  N  N  N  K  P  E  I  S  V  K  E  F  I  D  W  M  H>

      4890       4900       4910       4920       4930       4940       4950       4960
TGGAACCACA GTCCATGGTT TGGCTCCCAG TTTTACATCG AGTGGCAGCA GCGGAGACTG CAAAACATCA GGCCAAATGC
L  E  P  Q  S  M  V  W  L  P  V  L  H  R  V  A  A  A  E  T  A  K  H  Q  A  K  C>

      4970       4980       4990       5000       5010       5020       5030       5040
AACATCTGTA AAGAATGTCC AATTGTCGGG TTCAGGTATA GAAGCCTTAA GCATTTTAAC TATGATGTCT GCCAGAGTTG
  N  I  C  K  E  C  P  I  V  G  F  R  Y  R  S  L  K  H  F  N  Y  D  V  C  Q  S  C>

      5050       5060       5070       5080       5090       5100       5110       5120
TTTCTTTTCG GGTCGAACAG CAAAAGGTCA CAAATTACAT TACCCAATGG TGGAATATTG TATACCTACA ACATCTGGGG
  F  F  S  G  R  T  A  K  G  H  K  L  H  Y  P  M  V  E  Y  C  I  P  T  T  S  G>

      5130       5140       5150       5160       5170       5180       5190       5200
AAGATGTACG AGACTTCACA AAGGTACTTA AGAACAAGTT CAGGTCGAAG AAGTACTTTG CCAAACACCC TCGACTTGGT
E  D  V  R  D  F  T  K  V  L  K  N  K  F  R  S  K  K  Y  F  A  K  H  P  R  L  G>

      5210       5220       5230       5240       5250       5260       5270       5280
TACCTGCCTG TCCAGACAGT TCTTGAAGGT GACAACTTAG AGACTCCTAT CACACTCATC AGTATGTGGC CAGAGCACTA
Y  L  P  V  Q  T  V  L  E  G  D  N  L  E  T  P  I  T  L  I  S  M  W  P  E  H  Y>

      5290       5300       5310       5320       5330       5340       5350       5360
TGACCCCTCA CAATCTCCTC AACTGTTTCA TGATGACACC CATTCAAGAA TAGAACAATA TGCCACACGA CTGGCCCAGA
  D  P  S  Q  S  P  Q  L  F  H  D  D  T  H  S  R  I  E  Q  Y  A  T  R  L  A  Q>

      5370       5380       5390       5400       5410       5420       5430       5440
TGGAAAGGAC TAATGGGTCT TTTCTCACTG ATAGCAGCTC CACCACAGGA AGTGTGGAAG ACGAGCACGC CCTCATCCAG
```

Figure 9 cont ...

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M  E  R  T  N  G  S  F  L  T  D  S  S  S  T  T  G  S  V  E  D  E  H  A  L  I  Q>

      5450       5460       5470       5480       5490       5500       5510       5520
CAGTATTGCC AAACACTCGG AGGAGAGTCC CCAGTGAGCC AGCCGCAGAG CCCAGCTCAG ATCCTGAAGT CAGTAGAGAG
 Q  Y  C  Q  T  L  G  G  E  S  P  V  S  Q  P  Q  S  P  A  Q  I  L  K  S  V  E  R>

      5530       5540       5550       5560       5570       5580       5590       5600
GGAAGAACGT GGAGAACTGG AGAGGATCAT TGCTGACCTG GAGGAAGAAC AAAGAAATCT ACAGGTGGAG TATGAGCAGC
  E  E  R  G  E  L  E  R  I  I  A  D  L  E  E  E  Q  R  N  L  Q  V  E  Y  E  Q>

      5610       5620       5630       5640       5650       5660       5670       5680
TGAAGGACCA GCACCTCCGA AGGGGGCTCC CTGTCGGTTC ACCGCCAGAG TCGATTATAT CTCCCCATCA CACGTCTGAG
L  K  D  Q  H  L  R  R  G  L  P  V  G  S  P  P  E  S  I  I  S  P  H  H  T  S  E>

      5690       5700       5710       5720       5730       5740       5750       5760
GATTCAGAAC TTATAGCAGA AGCAAAACTC CTCAGGCAGC ACAAAGGTCG GCTGGAGGCT AGGATGCAGA TTTTAGAAGA
 D  S  E  L  I  A  E  A  K  L  L  R  Q  H  K  G  R  L  E  A  R  M  Q  I  L  E  D>

      5770       5780       5790       5800       5810       5820       5830       5840
TCACAATAAA CAGCTGGAGT CTCAGCTCCA CCGCCTCCGA CAGCTGCTGG AGCAGCCTGA ATCTGATTCC CGAATCAATG
  H  N  K  Q  L  E  S  Q  L  H  R  L  R  Q  L  L  E  Q  P  E  S  D  S  R  I  N>

      5850       5860       5870       5880       5890       5900       5910       5920
GTGTTTCCCC ATGGGCTTCT CCTCAGCATT CTGCACTGAG CTACTCGCTT GATCCAGATG CCTCCGGCCC ACAGTTCCAC
G  V  S  P  W  A  S  P  Q  H  S  A  L  S  Y  S  L  D  P  D  A  S  G  P  Q  F  H>

      5930       5940       5950       5960       5970       5980       5990       6000
CAGGCAGCGG GAGAGGACCT GCTGGCCCCA CCGCACGACA CCAGCACGGA TCTCACGGAG GTCATGGAGC AGATTCACAG
 Q  A  A  G  E  D  L  L  A  P  P  H  D  T  S  T  D  L  T  E  V  M  E  Q  I  H  S>

      6010       6020       6030       6040       6050
CACGTTTCCA TCTTGCTGCC CAAATGTTCC CAGCAGGCCA CAGGCAATGT AATCACTAG
  T  F  P  S  C  C  P  N  V  P  S  R  P  Q  A  M  *>
```
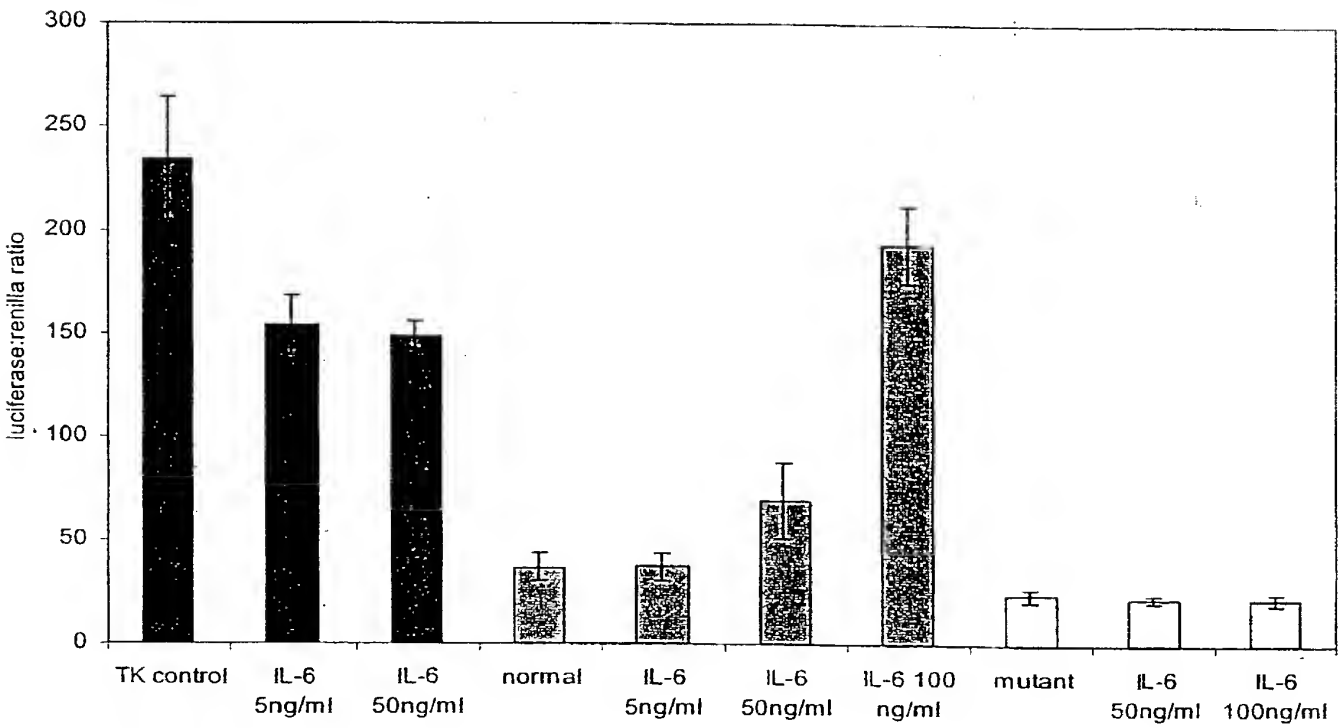

Figure 9 cont ...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250
200
150
100
50
0
luciferase:renilla ratio
TK control
IL-6 5ng/ml
IL-6 50ng/ml
normal
IL-6 5ng/ml
IL-6 50ng/ml
IL-6 100 ng/ml
mutant
IL-6 50ng/ml
IL-6 100ng/ml

Figure 10

INVENTOR DECLARATION	Attorney Docket No.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled , the specification of which

(check one) ☐ is attached hereto.

☒ was filed on as Application Serial No.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
9923423.9 (Number)	United Kingdom (Country)	4 October 1999 (Day/Month/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

(Application Serial No.) (Filing Date)

(Application Serial No.) (Filing Date)

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or § 365(b) of any PCT international application designating the United States of America, listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior U.S. or PCT international application in the manner provided by the first paragraph of Title 35, U.S.C. §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
PCT/GB 00/03800	4 October 2000	
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Application No. Docket No.

Please direct all correspondence regarding this application to the following:

FULBRIGHT & JAWORSKI L.L.P.
Attn: Melissa L. Sistrunk
1301 McKinney, Suite 5100
Houston, Texas 77010-3095
Telephone: (713) 651 3735
Fax: (713) 651-5246

For: Isis Innovation Limited

By: ..... Dated: 15-MAY-2002

Printed Name: .....

Mr. T. Hockaday
Executive Director
Isis Innovation Ltd

N.82592A

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EU110398085US in an envelope address to: Commissioner for Patents, Washington, DC 20231, on the date shown below

Dated: 8/27/02 Signature Staci V. Harris ~~(Susan Hunter)~~ Staci Harris

Docket No. (PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Isis Innovation Limited

Customer No.:

Application No.:

Group Art Unit:

Filed:

Examiner:

For: UTROPHIN GENE PROMOTER

POWER OF ATTORNEY

Commisioner for Patents
Washington, DC 20231

Dear Sir:

Isis Innovation Limited, assignee of the entire right, title and interest in the above-identified application by assignment dated ....., submitted herewith, hereby appoints the members of the firm of Fulbright & Jaworski L.L.P., a firm composed of:

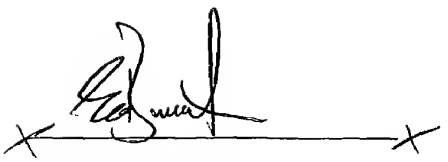
Melissa W. Acosta	45,872	Paul E. Krieger	25,886	John E. Schneider	31,998
Alberto Q. Amatong	41,580	Michael S. McCoy	46,913	Jan K. Simpson	33,283
Ronald G. Bliss	28,691	John M. Mings	35,955	Melissa L. Sistrunk	45,579
Gino Catena	45,546	Thomas D. Paul	32,714	Edward D. Steakley	47,964
Paul L. Deverter II	19,747	Jayne C. Piana	P-48,424	William A. Stout	18,773
Mark L. Delflache	28,942	Doak C. Procter, IV	P-48,705	Charles B. Walker, Jr.,	43,231
David L. Fox	40,612	James W. Repass	30,487	Richard S. Zembek	43,306
Eric B. Hall	46,751				

22

as its attorneys with full power of substitution to prosecute this application and to transact all business in the Patent and Trademark Office in connection therewith.

The assignee certifies that it has reviewed the assignment and to the best of the assignee's knowledge and belief, title is in the assignee.

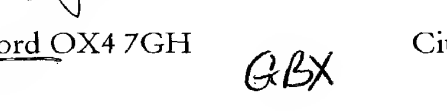
N.82592A

Name: Edward BURTON  Date: 16/4/02

1 00 Residence: University of Pittsburgh, School of Medicine, Department of Molecular Genetics and Biochemistry, E1215 Biomedical Science Tower, Pittsburgh, Pennsylvania 15261, United States of America

Citizenship: British PA

Post Office Address: University of Pittsburgh, School of Medicine, Department of Molecular Genetics and Biochemistry, E1215 Biomedical Science Tower, Pittsburgh, Pennsylvania 15261, United States of America

Name: Jonathan TINSLEY  Date: 15/4/02

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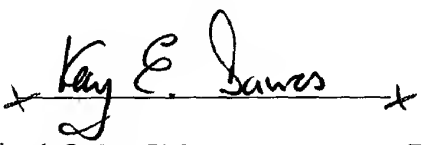
Name: Kay DAVIES  Date: 12/4/02

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SEQUENCE LISTING

<110> Burton, Edward
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Davies, Kay

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Thr Ser Leu Pro Lys Glu Arg Gly Ser Thr Arg Val His Ala Leu Asn
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Ser Val Arg Arg Leu Ala Ile Leu Lys Glu Asp Met Glu Met Lys Arg
575 580 585

cag act ctg gat caa ctg agt gag att ggc cag gat gtg ggc caa tta 1825
Gln Thr Leu Asp Gln Leu Ser Glu Ile Gly Gln Asp Val Gly Gln Leu
590 595 600 605

ctc agt aat ccc aag gca tct aag aag atg aac agt gac tct gag gag 1873
Leu Ser Asn Pro Lys Ala Ser Lys Lys Met Asn Ser Asp Ser Glu Glu
610 615 620

cta aca cag aga tgg gat tct ctg gtt cag aga ctc gaa gac tct tct 1921
Leu Thr Gln Arg Trp Asp Ser Leu Val Gln Arg Leu Glu Asp Ser Ser
625 630 635

aac cag gtg act cag gcg gta gcg aag ctc ggc atg tcc cag att cca 1969
Asn Gln Val Thr Gln Ala Val Ala Lys Leu Gly Met Ser Gln Ile Pro
640 645 650

cag aag gac cta ttg gag acc gtt cat gtg aga gaa caa ggg atg gtg 2017
Gln Lys Asp Leu Leu Glu Thr Val His Val Arg Glu Gln Gly Met Val
655 660 665

aag aag ccc aag cag gaa ctg cct cct ccg tta aca aag gct gag cat 2065
Lys Lys Pro Lys Gln Glu Leu Pro Pro Pro Leu Thr Lys Ala Glu His
670 675 680 685

gct atg caa aag aga tca acc acc gaa ttg gga gaa aac ctg caa gaa 2113
Ala Met Gln Lys Arg Ser Thr Thr Glu Leu Gly Glu Asn Leu Gln Glu
690 695 700

tta aga gac tta act caa gaa atg gaa gta cat gct gaa aaa ctc aaa 2161
Leu Arg Asp Leu Thr Gln Glu Met Glu Val His Ala Glu Lys Leu Lys
705 710 715

tgg ctg aat aga act gaa ttg gag atg ctt tca gat aaa agt ctg agt 2209
Trp Leu Asn Arg Thr Glu Leu Glu Met Leu Ser Asp Lys Ser Leu Ser
720 725 730

tta cct gaa agg gat aaa att tca gaa agc tta agg act gta aat atg 2257
Leu Pro Glu Arg Asp Lys Ile Ser Glu Ser Leu Arg Thr Val Asn Met
735 740 745

aca tgg aat aag att tgc aga gag gtg cct acc acc ctg aag gaa tgc 2305
Thr Trp Asn Lys Ile Cys Arg Glu Val Pro Thr Thr Leu Lys Glu Cys
750 755 760 765

atc cag gag ccc agt tct gtt tca cag aca agg att gct gct cat cct 2353
Ile Gln Glu Pro Ser Ser Val Ser Gln Thr Arg Ile Ala Ala His Pro

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                    770                 775                 780

aat gtc caa aag gtg gtg cta gta tca tct gcg tca gat att cct gtt   2401
Asn Val Gln Lys Val Val Leu Val Ser Ser Ala Ser Asp Ile Pro Val
            785                 790                 795

cag tct cat cgt act tcg gaa att tca att cct gct gat ctt gat aaa   2449
Gln Ser His Arg Thr Ser Glu Ile Ser Ile Pro Ala Asp Leu Asp Lys
        800                 805                 810

act ata aca gaa cta gcc gac tgg ctg gta tta atc gac cag atg ctg   2497
Thr Ile Thr Glu Leu Ala Asp Trp Leu Val Leu Ile Asp Gln Met Leu
    815                 820                 825

aag tcc aac att gtc act gtt ggg gat gta gaa gag atc aat aag acc   2545
Lys Ser Asn Ile Val Thr Val Gly Asp Val Glu Glu Ile Asn Lys Thr
830                 835                 840                 845

gtt tcc cga atg aaa att aca aag gct gac tta gaa cag cgc cat cct   2593
Val Ser Arg Met Lys Ile Thr Lys Ala Asp Leu Glu Gln Arg His Pro
                850                 855                 860

cag ctg gat tat gtt ttt aca ttg gca cag aat ttg aaa aat aaa gct   2641
Gln Leu Asp Tyr Val Phe Thr Leu Ala Gln Asn Leu Lys Asn Lys Ala
            865                 870                 875

tcc agt tca gat atg aga aca gca att aca gaa aaa ttg gaa agg gtc   2689
Ser Ser Ser Asp Met Arg Thr Ala Ile Thr Glu Lys Leu Glu Arg Val
        880                 885                 890

aag aac cag tgg gat ggc acc cag cat ggc gtt gag cta aga cag cag   2737
Lys Asn Gln Trp Asp Gly Thr Gln His Gly Val Glu Leu Arg Gln Gln
    895                 900                 905

cag ctt gag gac atg att att gac agt ctt cag tgg gat gac cat agg   2785
Gln Leu Glu Asp Met Ile Ile Asp Ser Leu Gln Trp Asp Asp His Arg
910                 915                 920                 925

gag gag act gaa gaa ctg atg aga aaa tat gag gct cga ctc tat att   2833
Glu Glu Thr Glu Glu Leu Met Arg Lys Tyr Glu Ala Arg Leu Tyr Ile
                930                 935                 940

ctt cag caa gcc cga cgg gat cca ctc acc aaa caa att tct gat aac   2881
Leu Gln Gln Ala Arg Arg Asp Pro Leu Thr Lys Gln Ile Ser Asp Asn
            945                 950                 955

caa ata ctg ctt caa gaa ctg ggt cct gga gat ggt atc gtc atg gcg   2929
Gln Ile Leu Leu Gln Glu Leu Gly Pro Gly Asp Gly Ile Val Met Ala
        960                 965                 970

ttc gat aac gtc ctg cag aaa ctc ctg gag gaa tat ggg agt gat gac   2977
Phe Asp Asn Val Leu Gln Lys Leu Leu Glu Glu Tyr Gly Ser Asp Asp
    975                 980                 985

aca agg aat gtg aaa gaa acc aca gag tac tta aaa aca tca tgg atc   3025
Thr Arg Asn Val Lys Glu Thr Thr Glu Tyr Leu Lys Thr Ser Trp Ile
990                 995                1000                1005

aat ctc aaa caa agt att gct gac aga cag aac gcc ttg gag gct gag   3073
Asn Leu Lys Gln Ser Ile Ala Asp Arg Gln Asn Ala Leu Glu Ala Glu
                1010                1015                1020
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tgg agg acg gtg cag gcc tct cgc aga gat ctg gaa aac ttc ctg aag   3121
Trp Arg Thr Val Gln Ala Ser Arg Arg Asp Leu Glu Asn Phe Leu Lys
            1025                1030                1035

tgg atc caa gaa gca gag acc aca gtg aat gtg ctt gtg gat gcc tct   3169
Trp Ile Gln Glu Ala Glu Thr Thr Val Asn Val Leu Val Asp Ala Ser
        1040                1045                1050

cat cgg gag aat gct ctt cag gat agt atc ttg gcc agg gaa ctc aaa   3217
His Arg Glu Asn Ala Leu Gln Asp Ser Ile Leu Ala Arg Glu Leu Lys
    1055                1060                1065

cag cag atg cag gac atc cag gca gaa att gat gcc cac aat gac ata   3265
Gln Gln Met Gln Asp Ile Gln Ala Glu Ile Asp Ala His Asn Asp Ile
1070                1075                1080                1085

ttt aaa agc att gac gga aac agg cag aag atg gta aaa gct ttg gga   3313
Phe Lys Ser Ile Asp Gly Asn Arg Gln Lys Met Val Lys Ala Leu Gly
                1090                1095                1100

aat tct gaa gag gct act atg ctt caa cat cga ctg gat gat atg aac   3361
Asn Ser Glu Glu Ala Thr Met Leu Gln His Arg Leu Asp Asp Met Asn
            1105                1110                1115

caa aga tgg aat gac tta aaa gca aaa tct gct agc atc agg gcc cat   3409
Gln Arg Trp Asn Asp Leu Lys Ala Lys Ser Ala Ser Ile Arg Ala His
        1120                1125                1130

ttg gag gcc agc gct gag aag tgg aac agg ttg ctg atg tcc tta gaa   3457
Leu Glu Ala Ser Ala Glu Lys Trp Asn Arg Leu Leu Met Ser Leu Glu
    1135                1140                1145

gaa ctg atc aaa tgg ctg aat atg aaa gat gaa gag ctt aag aaa caa   3505
Glu Leu Ile Lys Trp Leu Asn Met Lys Asp Glu Glu Leu Lys Lys Gln
1150                1155                1160                1165

atg cct att gga gga gat gtt cca gcc tta cag ctc cag tat gac cat   3553
Met Pro Ile Gly Gly Asp Val Pro Ala Leu Gln Leu Gln Tyr Asp His
                1170                1175                1180

tgt aag gcc ctg aga cgg gag tta aag gag aaa gaa tat tct gtc ctg   3601
Cys Lys Ala Leu Arg Arg Glu Leu Lys Glu Lys Glu Tyr Ser Val Leu
            1185                1190                1195

aat gct gtc gac cag gcc cga gtt ttc ttg gct gat cag cca att gag   3649
Asn Ala Val Asp Gln Ala Arg Val Phe Leu Ala Asp Gln Pro Ile Glu
        1200                1205                1210

gcc cct gaa gag cca aga aga aac cta caa tca aaa aca gaa tta act   3697
Ala Pro Glu Glu Pro Arg Arg Asn Leu Gln Ser Lys Thr Glu Leu Thr
    1215                1220                1225

cct gag gag aga gcc caa aag att gcc aaa gcc atg cgc aaa cag tct   3745
Pro Glu Glu Arg Ala Gln Lys Ile Ala Lys Ala Met Arg Lys Gln Ser
1230                1235                1240                1245

tct gaa gtc aaa gaa aaa tgg gaa agt cta aat gct gta act agc aat   3793
Ser Glu Val Lys Glu Lys Trp Glu Ser Leu Asn Ala Val Thr Ser Asn
                1250                1255                1260
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tgg caa aag caa gtg gac aag gca ttg gag aaa ctc aga gac ctg cag 3841
Trp Gln Lys Gln Val Asp Lys Ala Leu Glu Lys Leu Arg Asp Leu Gln
1265 1270 1275

gga gct atg gat gac ctg gac gct gac atg aag gag gca gag tcc gtg 3889
Gly Ala Met Asp Asp Leu Asp Ala Asp Met Lys Glu Ala Glu Ser Val
1280 1285 1290

cgg aat ggc tgg aag ccc gtg gga gac tta ctc att gac tcg ctg cag 3937
Arg Asn Gly Trp Lys Pro Val Gly Asp Leu Leu Ile Asp Ser Leu Gln
1295 1300 1305

gat cac att gaa aaa atc atg gca ttt aga gaa gaa att gca cca atc 3985
Asp His Ile Glu Lys Ile Met Ala Phe Arg Glu Glu Ile Ala Pro Ile
1310 1315 1320 1325

aac ttt aaa gtt aaa acg gtg aat gat tta tcc agt cag ctg tct cca 4033
Asn Phe Lys Val Lys Thr Val Asn Asp Leu Ser Ser Gln Leu Ser Pro
1330 1335 1340

ctt gac ctg cat ccc tct cta aag atg tct cgc cag cta gat gac ctt 4081
Leu Asp Leu His Pro Ser Leu Lys Met Ser Arg Gln Leu Asp Asp Leu
1345 1350 1355

aat atg cga tgg aaa ctt tta cag gtt tct gtg gat gat cgc ctt aaa 4129
Asn Met Arg Trp Lys Leu Leu Gln Val Ser Val Asp Asp Arg Leu Lys
1360 1365 1370

cag ctt cag gaa gcc cac aga gat ttt gga cca tcc tct cag cat ttt 4177
Gln Leu Gln Glu Ala His Arg Asp Phe Gly Pro Ser Ser Gln His Phe
1375 1380 1385

ctc tct acg tca gtc cag ctg ccg tgg caa aga tcc att tca cat aat 4225
Leu Ser Thr Ser Val Gln Leu Pro Trp Gln Arg Ser Ile Ser His Asn
1390 1395 1400 1405

aaa gtg ccc tat tac atc aac cat caa aca cag acc acc tgt tgg gac 4273
Lys Val Pro Tyr Tyr Ile Asn His Gln Thr Gln Thr Thr Cys Trp Asp
1410 1415 1420

cat cct aaa atg acc gaa ctc ttt caa tcc ctt gct gac ctg aat aat 4321
His Pro Lys Met Thr Glu Leu Phe Gln Ser Leu Ala Asp Leu Asn Asn
1425 1430 1435

gta cgt ttt tct gcc tac cgt aca gca atc aaa atc cga aga cta caa 4369
Val Arg Phe Ser Ala Tyr Arg Thr Ala Ile Lys Ile Arg Arg Leu Gln
1440 1445 1450

aaa gca cta tgt ttg gat ctc tta gag ttg agt aca aca aat gaa att 4417
Lys Ala Leu Cys Leu Asp Leu Leu Glu Leu Ser Thr Thr Asn Glu Ile
1455 1460 1465

ttc aaa cag cac aag ttg aac caa aat gac cag ctc ctc agt gtt cca 4465
Phe Lys Gln His Lys Leu Asn Gln Asn Asp Gln Leu Leu Ser Val Pro
1470 1475 1480 1485

gat gtc atc aac tgt ctg aca aca act tat gat gga ctt gag caa atg 4513
Asp Val Ile Asn Cys Leu Thr Thr Thr Tyr Asp Gly Leu Glu Gln Met
1490 1495 1500

cat aag gac ctg gtc aac gtt cca ctc tgt gtt gat atg tgt ctc aat 4561

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His Lys Asp Leu Val Asn Val Pro Leu Cys Val Asp Met Cys Leu Asn
          1505                1510                1515

tgg ttg ctc aat gtc tat gac acg ggt cga act gga aaa att aga gtg   4609
Trp Leu Leu Asn Val Tyr Asp Thr Gly Arg Thr Gly Lys Ile Arg Val
      1520                1525                1530

cag agt ctg aag att gga tta atg tct ctc tcc aaa ggt ctc ttg gaa   4657
Gln Ser Leu Lys Ile Gly Leu Met Ser Leu Ser Lys Gly Leu Leu Glu
   1535                1540                1545

gaa aaa tac aga tat ctc ttt aag gaa gtt gcg ggg ccg aca gaa atg   4705
Glu Lys Tyr Arg Tyr Leu Phe Lys Glu Val Ala Gly Pro Thr Glu Met
1550                1555                1560                1565

tgt gac cag agg cag ctg ggc ctg tta ctt cat gat gcc atc cag atc   4753
Cys Asp Gln Arg Gln Leu Gly Leu Leu Leu His Asp Ala Ile Gln Ile
               1570                1575                1580

ccc cgg cag cta ggt gaa gta gca gct ttt gga ggc agt aat att gag   4801
Pro Arg Gln Leu Gly Glu Val Ala Ala Phe Gly Gly Ser Asn Ile Glu
          1585                1590                1595

cct agt gtt cgc agc tgc ttc caa cag aat aac aat aaa cca gaa ata   4849
Pro Ser Val Arg Ser Cys Phe Gln Gln Asn Asn Asn Lys Pro Glu Ile
      1600                1605                1610

agt gtg aaa gag ttt ata gat tgg atg cat ttg gaa cca cag tcc atg   4897
Ser Val Lys Glu Phe Ile Asp Trp Met His Leu Glu Pro Gln Ser Met
   1615                1620                1625

gtt tgg ctc cca gtt tta cat cga gtg gca gca gcg gag act gca aaa   4945
Val Trp Leu Pro Val Leu His Arg Val Ala Ala Ala Glu Thr Ala Lys
1630                1635                1640                1645

cat cag gcc aaa tgc aac atc tgt aaa gaa tgt cca att gtc ggg ttc   4993
His Gln Ala Lys Cys Asn Ile Cys Lys Glu Cys Pro Ile Val Gly Phe
               1650                1655                1660

agg tat aga agc ctt aag cat ttt aac tat gat gtc tgc cag agt tgt   5041
Arg Tyr Arg Ser Leu Lys His Phe Asn Tyr Asp Val Cys Gln Ser Cys
          1665                1670                1675

ttc ttt tcg ggt cga aca gca aaa ggt cac aaa tta cat tac cca atg   5089
Phe Phe Ser Gly Arg Thr Ala Lys Gly His Lys Leu His Tyr Pro Met
      1680                1685                1690

gtg gaa tat tgt ata cct aca aca tct ggg gaa gat gta cga gac ttc   5137
Val Glu Tyr Cys Ile Pro Thr Thr Ser Gly Glu Asp Val Arg Asp Phe
   1695                1700                1705

aca aag gta ctt aag aac aag ttc agg tcg aag aag tac ttt gcc aaa   5185
Thr Lys Val Leu Lys Asn Lys Phe Arg Ser Lys Lys Tyr Phe Ala Lys
1710                1715                1720                1725

cac cct cga ctt ggt tac ctg cct gtc cag aca gtt ctt gaa ggt gac   5233
His Pro Arg Leu Gly Tyr Leu Pro Val Gln Thr Val Leu Glu Gly Asp
               1730                1735                1740

aac tta gag act cct atc aca ctc atc agt atg tgg cca gag cac tat   5281
Asn Leu Glu Thr Pro Ile Thr Leu Ile Ser Met Trp Pro Glu His Tyr
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1745 1750 1755

gac ccc tca caa tct cct caa ctg ttt cat gat gac acc cat tca aga 5329
Asp Pro Ser Gln Ser Pro Gln Leu Phe His Asp Asp Thr His Ser Arg
1760 1765 1770

ata gaa caa tat gcc aca cga ctg gcc cag atg gaa agg act aat ggg 5377
Ile Glu Gln Tyr Ala Thr Arg Leu Ala Gln Met Glu Arg Thr Asn Gly
1775 1780 1785

tct ttt ctc act gat agc agc tcc acc aca gga agt gtg gaa gac gag 5425
Ser Phe Leu Thr Asp Ser Ser Ser Thr Thr Gly Ser Val Glu Asp Glu
1790 1795 1800 1805

cac gcc ctc atc cag cag tat tgc caa aca ctc gga gga gag tcc cca 5473
His Ala Leu Ile Gln Gln Tyr Cys Gln Thr Leu Gly Gly Glu Ser Pro
1810 1815 1820

gtg agc cag ccg cag agc cca gct cag atc ctg aag tca gta gag agg 5521
Val Ser Gln Pro Gln Ser Pro Ala Gln Ile Leu Lys Ser Val Glu Arg
1825 1830 1835

gaa gaa cgt gga gaa ctg gag agg atc att gct gac ctg gag gaa gaa 5569
Glu Glu Arg Gly Glu Leu Glu Arg Ile Ile Ala Asp Leu Glu Glu Glu
1840 1845 1850

caa aga aat cta cag gtg gag tat gag cag ctg aag gac cag cac ctc 5617
Gln Arg Asn Leu Gln Val Glu Tyr Glu Gln Leu Lys Asp Gln His Leu
1855 1860 1865

cga agg ggg ctc cct gtc ggt tca ccg cca gag tcg att ata tct ccc 5665
Arg Arg Gly Leu Pro Val Gly Ser Pro Pro Glu Ser Ile Ile Ser Pro
1870 1875 1880 1885

cat cac acg tct gag gat tca gaa ctt ata gca gaa gca aaa ctc ctc 5713
His His Thr Ser Glu Asp Ser Glu Leu Ile Ala Glu Ala Lys Leu Leu
1890 1895 1900

agg cag cac aaa ggt cgg ctg gag gct agg atg cag att tta gaa gat 5761
Arg Gln His Lys Gly Arg Leu Glu Ala Arg Met Gln Ile Leu Glu Asp
1905 1910 1915

cac aat aaa cag ctg gag tct cag ctc cac cgc ctc cga cag ctg ctg 5809
His Asn Lys Gln Leu Glu Ser Gln Leu His Arg Leu Arg Gln Leu Leu
1920 1925 1930

gag cag cct gaa tct gat tcc cga atc aat ggt gtt tcc cca tgg gct 5857
Glu Gln Pro Glu Ser Asp Ser Arg Ile Asn Gly Val Ser Pro Trp Ala
1935 1940 1945

tct cct cag cat tct gca ctg agc tac tcg ctt gat cca gat gcc tcc 5905
Ser Pro Gln His Ser Ala Leu Ser Tyr Ser Leu Asp Pro Asp Ala Ser
1950 1955 1960 1965

ggc cca cag ttc cac cag gca gcg gga gag gac ctg ctg gcc cca ccg 5953
Gly Pro Gln Phe His Gln Ala Ala Gly Glu Asp Leu Leu Ala Pro Pro
1970 1975 1980

cac gac acc agc acg gat ctc acg gag gtc atg gag cag att cac agc 6001
His Asp Thr Ser Thr Asp Leu Thr Glu Val Met Glu Gln Ile His Ser
1985 1990 1995

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acg ttt cca tct tgc tgc cca aat gtt ccc agc agg cca cag gca atg   6049
Thr Phe Pro Ser Cys Cys Pro Asn Val Pro Ser Arg Pro Gln Ala Met
       2000                2005                2010

taa tcactag                                                      6059
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<210> 9
<211> 2013
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted amino acid sequence of a utrophin B isoform "minigene"

<400> 9

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Asp Leu Pro Gly His Val Ala Leu Gln Ala Cys Lys Arg Leu Pro Asp
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Glu His Asn Asp Val Gln Lys Lys Thr Phe Thr Lys Trp Ile Asn Ala
         35                  40                  45
Arg Phe Ser Lys Ser Gly Lys Pro Pro Ile Ser Asp Met Phe Ser Asp
     50                  55                  60
Leu Lys Asp Gly Arg Lys Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly
 65                  70                  75                  80
Thr Ser Leu Pro Lys Glu Arg Gly Ser Thr Arg Val His Ala Leu Asn
                 85                  90                  95
Asn Val Asn Arg Val Leu Gln Val Leu His Gln Asn Asn Val Asp Leu
            100                 105                 110
Val Asn Ile Gly Gly Thr Asp Ile Val Ala Gly Asn Pro Lys Leu Thr
        115                 120                 125
Leu Gly Leu Leu Trp Ser Ile Ile Leu His Trp Gln Val Lys Asp Val
    130                 135                 140
Met Lys Asp Ile Met Ser Asp Leu Gln Gln Thr Asn Ser Glu Lys Ile
145                 150                 155                 160
Leu Leu Ser Trp Val Arg Gln Thr Thr Arg Pro Tyr Ser Gln Val Asn
                165                 170                 175
Val Leu Asn Phe Thr Thr Ser Trp Thr Asp Gly Leu Ala Phe Asn Ala
            180                 185                 190
Val Leu His Arg His Lys Pro Asp Leu Phe Asp Trp Asp Glu Met Val
        195                 200                 205
Lys Met Ser Pro Ile Glu Arg Leu Asp His Ala Phe Asp Lys Ala His
    210                 215                 220
Thr Ser Leu Gly Ile Glu Lys Leu Leu Ser Pro Glu Thr Val Ala Val
225                 230                 235                 240
His Leu Pro Asp Lys Lys Ser Ile Ile Met Tyr Leu Thr Ser Leu Phe
                245                 250                 255
Glu Val Leu Pro Gln Gln Val Thr Ile Asp Ala Ile Arg Glu Val Glu
            260                 265                 270
Thr Leu Pro Arg Lys Tyr Lys Lys Glu Cys Glu Glu Glu Glu Ile His
        275                 280                 285
Ile Gln Ser Ala Val Leu Ala Glu Glu Gly Gln Ser Pro Arg Ala Glu
    290                 295                 300
Thr Pro Ser Thr Val Thr Glu Val Asp Met Asp Leu Asp Ser Tyr Gln
305                 310                 315                 320
Ile Ala Leu Glu Glu Val Leu Thr Trp Leu Leu Ser Ala Glu Asp Thr
                325                 330                 335
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Phe Gln Glu Gln His Asp Ile Ser Asp Asp Val Glu Glu Val Lys Glu
            340                 345                 350
Gln Phe Ala Thr His Glu Thr Phe Met Met Glu Leu Thr Ala His Gln
        355                 360                 365
Ser Ser Val Gly Ser Val Leu Gln Ala Gly Asn Gln Leu Met Thr Gln
    370                 375                 380
Gly Thr Leu Ser Arg Glu Glu Glu Phe Glu Ile Gln Glu Gln Met Thr
385                 390                 395                 400
Leu Leu Asn Ala Arg Trp Glu Ala Leu Arg Val Glu Ser Met Glu Arg
                405                 410                 415
Gln Ser Arg Leu His Asp Ala Leu Met Glu Leu Gln Lys Lys Gln Leu
            420                 425                 430
Gln Gln Leu Ser Ser Trp Leu Ala Leu Thr Glu Glu Arg Ile Gln Lys
        435                 440                 445
Met Glu Ser Leu Pro Leu Gly Asp Asp Leu Pro Ser Leu Gln Lys Leu
    450                 455                 460
Leu Gln Glu His Lys Ser Leu Gln Asn Asp Leu Glu Ala Glu Gln Val
465                 470                 475                 480
Lys Val Asn Ser Leu Thr His Met Val Val Ile Val Asp Glu Asn Ser
                485                 490                 495
Gly Glu Ser Ala Thr Ala Leu Leu Glu Asp Gln Leu Gln Lys Leu Gly
            500                 505                 510
Glu Arg Trp Thr Ala Val Cys Arg Trp Thr Glu Glu Arg Trp Asn Arg
        515                 520                 525
Leu Gln Glu Ile Ser Ile Leu Trp Gln Glu Leu Leu Glu Glu Gln Cys
    530                 535                 540
Leu Leu Glu Ala Trp Leu Thr Glu Lys Glu Glu Ala Leu Asp Lys Val
545                 550                 555                 560
Gln Thr Ser Asn Phe Lys Asp Gln Lys Glu Leu Ser Val Ser Val Arg
                565                 570                 575
Arg Leu Ala Ile Leu Lys Glu Asp Met Glu Met Lys Arg Gln Thr Leu
            580                 585                 590
Asp Gln Leu Ser Glu Ile Gly Gln Asp Val Gly Gln Leu Leu Ser Asn
        595                 600                 605
Pro Lys Ala Ser Lys Lys Met Asn Ser Asp Ser Glu Glu Leu Thr Gln
    610                 615                 620
Arg Trp Asp Ser Leu Val Gln Arg Leu Glu Asp Ser Ser Asn Gln Val
625                 630                 635                 640
Thr Gln Ala Val Ala Lys Leu Gly Met Ser Gln Ile Pro Gln Lys Asp
                645                 650                 655
Leu Leu Glu Thr Val His Val Arg Glu Gln Gly Met Val Lys Lys Pro
            660                 665                 670
Lys Gln Glu Leu Pro Pro Pro Leu Thr Lys Ala Glu His Ala Met Gln
        675                 680                 685
Lys Arg Ser Thr Thr Glu Leu Gly Glu Asn Leu Gln Glu Leu Arg Asp
    690                 695                 700
Leu Thr Gln Glu Met Glu Val His Ala Glu Lys Leu Lys Trp Leu Asn
705                 710                 715                 720
Arg Thr Glu Leu Glu Met Leu Ser Asp Lys Ser Leu Ser Leu Pro Glu
                725                 730                 735
Arg Asp Lys Ile Ser Glu Ser Leu Arg Thr Val Asn Met Thr Trp Asn
            740                 745                 750
Lys Ile Cys Arg Glu Val Pro Thr Thr Leu Lys Glu Cys Ile Gln Glu
        755                 760                 765
Pro Ser Ser Val Ser Gln Thr Arg Ile Ala Ala His Pro Asn Val Gln
    770                 775                 780
Lys Val Val Leu Val Ser Ser Ala Ser Asp Ile Pro Val Gln Ser His
785                 790                 795                 800
Arg Thr Ser Glu Ile Ser Ile Pro Ala Asp Leu Asp Lys Thr Ile Thr
                805                 810                 815
Glu Leu Ala Asp Trp Leu Val Leu Ile Asp Gln Met Leu Lys Ser Asn
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            820                 825                 830
Ile Val Thr Val Gly Asp Val Glu Glu Ile Asn Lys Thr Val Ser Arg
        835                 840                 845
Met Lys Ile Thr Lys Ala Asp Leu Glu Gln Arg His Pro Gln Leu Asp
    850                 855                 860
Tyr Val Phe Thr Leu Ala Gln Asn Leu Lys Asn Lys Ala Ser Ser Ser
865                 870                 875                 880
Asp Met Arg Thr Ala Ile Thr Glu Lys Leu Glu Arg Val Lys Asn Gln
                885                 890                 895
Trp Asp Gly Thr Gln His Gly Val Glu Leu Arg Gln Gln Gln Leu Glu
            900                 905                 910
Asp Met Ile Ile Asp Ser Leu Gln Trp Asp Asp His Arg Glu Glu Thr
        915                 920                 925
Glu Glu Leu Met Arg Lys Tyr Glu Ala Arg Leu Tyr Ile Leu Gln Gln
    930                 935                 940
Ala Arg Arg Asp Pro Leu Thr Lys Gln Ile Ser Asp Asn Gln Ile Leu
945                 950                 955                 960
Leu Gln Glu Leu Gly Pro Gly Asp Gly Ile Val Met Ala Phe Asp Asn
                965                 970                 975
Val Leu Gln Lys Leu Leu Glu Glu Tyr Gly Ser Asp Asp Thr Arg Asn
            980                 985                 990
Val Lys Glu Thr Thr Glu Tyr Leu Lys Thr Ser Trp Ile Asn Leu Lys
        995                1000                1005
Gln Ser Ile Ala Asp Arg Gln Asn Ala Leu Glu Ala Glu Trp Arg Thr
   1010                1015                1020
Val Gln Ala Ser Arg Arg Asp Leu Glu Asn Phe Leu Lys Trp Ile Gln
1025                1030                1035                1040
Glu Ala Glu Thr Thr Val Asn Val Leu Val Asp Ala Ser His Arg Glu
                1045                1050                1055
Asn Ala Leu Gln Asp Ser Ile Leu Ala Arg Glu Leu Lys Gln Gln Met
            1060                1065                1070
Gln Asp Ile Gln Ala Glu Ile Asp Ala His Asn Asp Ile Phe Lys Ser
        1075                1080                1085
Ile Asp Gly Asn Arg Gln Lys Met Val Lys Ala Leu Gly Asn Ser Glu
   1090                1095                1100
Glu Ala Thr Met Leu Gln His Arg Leu Asp Asp Met Asn Gln Arg Trp
1105                1110                1115                1120
Asn Asp Leu Lys Ala Lys Ser Ala Ser Ile Arg Ala His Leu Glu Ala
                1125                1130                1135
Ser Ala Glu Lys Trp Asn Arg Leu Leu Met Ser Leu Glu Glu Leu Ile
            1140                1145                1150
Lys Trp Leu Asn Met Lys Asp Glu Glu Leu Lys Lys Gln Met Pro Ile
        1155                1160                1165
Gly Gly Asp Val Pro Ala Leu Gln Leu Gln Tyr Asp His Cys Lys Ala
   1170                1175                1180
Leu Arg Arg Glu Leu Lys Glu Lys Glu Tyr Ser Val Leu Asn Ala Val
1185                1190                1195                1200
Asp Gln Ala Arg Val Phe Leu Ala Asp Gln Pro Ile Glu Ala Pro Glu
                1205                1210                1215
Glu Pro Arg Arg Asn Leu Gln Ser Lys Thr Glu Leu Thr Pro Glu Glu
            1220                1225                1230
Arg Ala Gln Lys Ile Ala Lys Ala Met Arg Lys Gln Ser Ser Glu Val
        1235                1240                1245
Lys Glu Lys Trp Glu Ser Leu Asn Ala Val Thr Ser Asn Trp Gln Lys
   1250                1255                1260
Gln Val Asp Lys Ala Leu Glu Lys Leu Arg Asp Leu Gln Gly Ala Met
1265                1270                1275                1280
Asp Asp Leu Asp Ala Asp Met Lys Glu Ala Glu Ser Val Arg Asn Gly
                1285                1290                1295
Trp Lys Pro Val Gly Asp Leu Leu Ile Asp Ser Leu Gln Asp His Ile
            1300                1305                1310
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Glu Lys Ile Met Ala Phe Arg Glu Glu Ile Ala Pro Ile Asn Phe Lys
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Val Lys Thr Val Asn Asp Leu Ser Ser Gln Leu Ser Pro Leu Asp Leu
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His Pro Ser Leu Lys Met Ser Arg Gln Leu Asp Asp Leu Asn Met Arg
1345                1350                1355                1360
Trp Lys Leu Leu Gln Val Ser Val Asp Asp Arg Leu Lys Gln Leu Gln
                1365                1370                1375
Glu Ala His Arg Asp Phe Gly Pro Ser Ser Gln His Phe Leu Ser Thr
            1380                1385                1390
Ser Val Gln Leu Pro Trp Gln Arg Ser Ile Ser His Asn Lys Val Pro
        1395                1400                1405
Tyr Tyr Ile Asn His Gln Thr Gln Thr Thr Cys Trp Asp His Pro Lys
    1410                1415                1420
Met Thr Glu Leu Phe Gln Ser Leu Ala Asp Leu Asn Asn Val Arg Phe
1425                1430                1435                1440
Ser Ala Tyr Arg Thr Ala Ile Lys Ile Arg Arg Leu Gln Lys Ala Leu
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Cys Leu Asp Leu Leu Glu Leu Ser Thr Thr Asn Glu Ile Phe Lys Gln
            1460                1465                1470
His Lys Leu Asn Gln Asn Asp Gln Leu Leu Ser Val Pro Asp Val Ile
        1475                1480                1485
Asn Cys Leu Thr Thr Thr Tyr Asp Gly Leu Glu Gln Met His Lys Asp
    1490                1495                1500
Leu Val Asn Val Pro Leu Cys Val Asp Met Cys Leu Asn Trp Leu Leu
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Asn Val Tyr Asp Thr Gly Arg Thr Gly Lys Ile Arg Val Gln Ser Leu
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Lys Ile Gly Leu Met Ser Leu Ser Lys Gly Leu Leu Glu Glu Lys Tyr
            1540                1545                1550
Arg Tyr Leu Phe Lys Glu Val Ala Gly Pro Thr Glu Met Cys Asp Gln
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Arg Gln Leu Gly Leu Leu Leu His Asp Ala Ile Gln Ile Pro Arg Gln
    1570                1575                1580
Leu Gly Glu Val Ala Ala Phe Gly Gly Ser Asn Ile Glu Pro Ser Val
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Arg Ser Cys Phe Gln Gln Asn Asn Asn Lys Pro Glu Ile Ser Val Lys
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Glu Phe Ile Asp Trp Met His Leu Glu Pro Gln Ser Met Val Trp Leu
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Pro Val Leu His Arg Val Ala Ala Ala Glu Thr Ala Lys His Gln Ala
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Lys Cys Asn Ile Cys Lys Glu Cys Pro Ile Val Gly Phe Arg Tyr Arg
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Ser Leu Lys His Phe Asn Tyr Asp Val Cys Gln Ser Cys Phe Phe Ser
1665                1670                1675                1680
Gly Arg Thr Ala Lys Gly His Lys Leu His Tyr Pro Met Val Glu Tyr
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Cys Ile Pro Thr Thr Ser Gly Glu Asp Val Arg Asp Phe Thr Lys Val
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Leu Lys Asn Lys Phe Arg Ser Lys Lys Tyr Phe Ala Lys His Pro Arg
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Leu Gly Tyr Leu Pro Val Gln Thr Val Leu Glu Gly Asp Asn Leu Glu
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Thr Pro Ile Thr Leu Ile Ser Met Trp Pro Glu His Tyr Asp Pro Ser
1745                1750                1755                1760
Gln Ser Pro Gln Leu Phe His Asp Asp Thr His Ser Arg Ile Glu Gln
                1765                1770                1775
Tyr Ala Thr Arg Leu Ala Gln Met Glu Arg Thr Asn Gly Ser Phe Leu
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Thr Asp Ser Ser Ser Thr Thr Gly Ser Val Glu Asp Glu His Ala Leu
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Pro Gln Ser Pro Ala Gln Ile Leu Lys Ser Val Glu Arg Glu Glu Arg
1825                1830                1835                1840
Gly Glu Leu Glu Arg Ile Ile Ala Asp Leu Glu Glu Glu Gln Arg Asn
                1845                1850                1855
Leu Gln Val Glu Tyr Glu Gln Leu Lys Asp Gln His Leu Arg Arg Gly
            1860                1865                1870
Leu Pro Val Gly Ser Pro Pro Glu Ser Ile Ile Ser Pro His His Thr
       1875                1880                1885
Ser Glu Asp Ser Glu Leu Ile Ala Glu Ala Lys Leu Leu Arg Gln His
   1890                1895                1900
Lys Gly Arg Leu Glu Ala Arg Met Gln Ile Leu Glu Asp His Asn Lys
1905                1910                1915                1920
Gln Leu Glu Ser Gln Leu His Arg Leu Arg Gln Leu Leu Glu Gln Pro
                1925                1930                1935
Glu Ser Asp Ser Arg Ile Asn Gly Val Ser Pro Trp Ala Ser Pro Gln
            1940                1945                1950
His Ser Ala Leu Ser Tyr Ser Leu Asp Pro Asp Ala Ser Gly Pro Gln
       1955                1960                1965
Phe His Gln Ala Ala Gly Glu Asp Leu Leu Ala Pro Pro His Asp Thr
   1970                1975                1980
Ser Thr Asp Leu Thr Glu Val Met Glu Gln Ile His Ser Thr Phe Pro
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Ser Cys Cys Pro Asn Val Pro Ser Arg Pro Gln Ala Met
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